ELECTRON MICROSCOPY OF THE CARDIOVASCULAR SYSTEM

REVISED AND ENLARGED ENGLISH EDITION

ELECTRON MICROSCOPY OF THE CARDIOVASCULAR SYSTEM

An Electron Microscopic Study with Applications to Physiology

By

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Translated from the Original German Text

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To

Dr GUSTAV J MARTIN

who has steadfastly followed the progress of the present study with the greatest of under standing and helpfulness this volume is dedi cated in fond admiration

PREFACE

Over the past ten years the author of this volume has been occupied with electron microscopic studies of the ultra structure of the heart and blood vessels. The results of these studies have been published in various English, French, Italian and German scientific journals. A small summation of the author's results up to that time appeared in book form in New York in 1951 and a monograph in 1957 (1957g) in Germany. Since 1951 the methods of electron microscopic study, especially fixation and embedding of specimens, the use of plastic as an embedding medium and last but not least methods for obtaining ultra thin sections have been changed so completely that the present series of pictures made in continuation of the aforementioned studies are hardly comparable at all with those pictures published back in 1951. The photographs of recent years allow the recognition of previously unsuspected details, structures 100 Å or less in size being clearly visible. Thus we are now afforded a previously unhoped for widening of our horizons and an altogether new understanding of the ultra structure of tissues and their functions.

A principal prerequisite of all physiology is the recognition of the structure of organs and tissues if one wishes to understand their functions. This becomes especially apparent when one looks for example at the ultramicroscopic structure of capillaries and realizes that this structure is the keystone of countless physiological and pathological processes which cannot be understood without first understanding the exact make up of the capillary wall.

The specific cell type found in the lungs which was described by Schlipköter (1954) and independently by this author in 1955 on the basis of electron microscopic studies has now been well substantiated. It points to a new function of the lung unknown until now, even though these cells are as yet not clearly understood as to their physiological significance.

In regard to the heart, electron microscopy has provided the answer or at least pointed to the final answer, to the age old question of why heart muscle can withstand a lifelong span of almost continuous work It has also yielded new aspects for the mechanism of angina pectoris and of axon reflexes

These few examples explain why, working in electron microscopy one comes to feel that the electron microscope will very soon be as vital to the future development of physiology pathology, and indeed every branch of biology, as the old light microscope was to the nineteenth century development of these sciences

This little volume attempts to show in a limited area what the new method of electron microscopy has contributed thus far to *the science of cardiology The presentation is based almost wholly* on the author s own research since, unfortunately his own pioneering work has to date, attracted but few co workers and associates in this field

The technical difficulties of electron microscopy are very great even today and as always in histology one must face the question of just how much of the observed material is actual and natural structure and how much is artifact produced by technical inadequacies The danger of the artifact which beguiles our judgment must not be underrated On the other hand fear of misinterpretation must not be allowed to stand in the way of progress To give an example from my own experience the great number of sarcosomes in the cardiac muscle of an animal when compared with its skeletal muscle (B Kisch 1950) could never have been an artifact, even if the pictures of the heart obtainable nine years ago were much less creditable than those taken today The important advance to cardiology of recognizing the sarcosomes as vital parts of cardiac muscle fibers (B Kisch 1952a) was *achieved on the basis of those early and very poor pictures* The importance of sarcosomes to cardiology is of course being worked out more fully today by means of the much better pictures now available Still today and even in the field of light microscopy artifacts play an important part In the histology of the last 150 years they have led to many *major discoveries After all every* stain is artificial and any method of fixation and embedding leads to falsified pictures

An example of this from electron microscopy is the following: increased periods of fixation in formalin, inadequate embedding in plastic and other such "mistakes" produce a rupture of the ultramicroscopic webbings and holes between the organelles. This is of course undesirable and our technique must try to avoid such errors. However only a dull "artifactophobe" would discard such pictures as unusable, failing to recognize that these very mistakes provide us with an ultramicroscopic teasing technique for the recognition of the finest organelles, similar to the teasing techniques which proved so useful to light microscopy 100 years ago. Therefore purposely, some of these pictures were still incorporated in this book. Here as everywhere the advancement of science requires both good technique and a critical mind.

It must be emphasized that this volume still does not pretend to be a definitive monograph on a well-defined subject. Rather it is a summarizing presentation of work done to date, a *map with* many blank areas of unexplored territory, to serve as a statement of that which we hope already to comprehend and a pointer to the far greater area about which we know nothing and which, as yet, remains to be explored.

The author has the pleasant duty of thanking those who helped him with the performance of these studies.

Above all he must thank the small group of the 1952 trustees of the American College of Cardiology (New York) who unanimously voted a large sum to buy an RCA electron microscope and to establish the Electron Microscope Research Institute, then the greatest achievement of the American College of Cardiology, solely to pursue the search for knowledge about the circulatory apparatus and its problems, both theoretical and clinical. In this respect the Institute is still unique. The author had the honor of being entrusted with the directorship of this Institute, now in possession of and sponsored by City Hospital at Elmhurst, New York. For two years the National Heart Institute of the National Institutes of Health (Public Health Service) in Washington supported this research with a grant (H 1489R).

In the following three years the research was made possible partly through generous grants of the National Drug Company in Philadelphia. The awarding of these grants was primarily due

to the interest and understanding of the firm's Vice President and director of research, Dr Gustav J Martin

Despite this help however the author was forced lacking any other support, to personally bear the heavy burden of a great part of the research expenses Without great personal sacrifices he would have been compelled to abandon these investigations many years ago It was only in 1959 that he again received a grant from the National Institutes of Health (H-4094)

Special thanks also go to Brooklyn Medical Press in New York for generously permitting the use of pictures already published in the journal *Experimental Medicine and Surgery* and, last but not least, Charles C Thomas Publisher for prompt and careful execution of the printing of this work

BRUNO KISCH

New York

CONTENTS

ELECTRON MICROSCOPY OF THE CARDIOVASCULAR SYSTEM

METHOD OF INVESTIGATION

Details of the construction and theory of the electron microscope will not be considered here Readers interested in these details are referred to the many special and technical publications on the electron microscope Readers interested only in a bird eye view of the inner workings of an electron microscope are referred to the excellent readable description by Bodo von Borries in *Gluckauf* (1955) and also to the article by the same author in *Radex Rundschau* (1956) or the book by Wyckoff (1949)

Basically the electron microscope operates on the same principle of ray refraction as does the light microscope Instead of refracting light rays however it refracts cathode rays consisting of free electrons

The idea of substituting cathode rays for light rays mainly the work of Bodo von Borries and E Ruska has radically changed the whole science of microscopy Objects of previously invisible size were now brought into the sphere of our visual acquaintance Light microscopy at its best had advanced the limits of visible objects to a size of about 0 25 μ The ingenious mathematical and physical studies of Ernst Abbe and his painstaking research into all the materials that could be used in a light microscope as light refracting media paved the way for the triumphs achieved through light microscope research Light microscopes however have a set finite limit which is determined not only by the instrument itself but by the wave length of the rays it employs Abbe and other physicists demonstrated the fact that two separate particles can be seen as separate objects only when the distance between them is at least one half the wave length of the rays used to visualize them This is the reason why microscopists have on

occasion substituted the shorter waved ultraviolet light for the ordinary white light By doing this, they could increase somewhat the resolving power of the light microscope, and see some small otherwise invisible objects Visible light has a wave length of approximately one half of one micron, and this determines the fact that the usual light microscopes have a maximal magnification power of only 1500×

The magnifications possible when cathode rays are substituted for visible light rays are of a quite different order Electrons radiating out from a piece of red hot metal can be directed at high speeds toward an anode in an electric field According to von Borries such a beam of electrons, driven from a heated cathode toward an anode in an electric field of 60,000 volts achieves a speed approximately one half that of the speed of light Electrons are the lightest particles known today The wave length of these minute particles is only a tiny fraction (according to von Borries 1/100 000) of the wave length of visible light

These basic facts made it at once apparent to the first pioneers of the idea of an electron microscope that here was a method whereby a new world existing, but as yet invisible, could at last be made visible to the eye These expectations have already been realized to some small extent Electron microscopes today manufactured by various firms the world over such as Siemens and Halske in Germany Phillips in Holland and RCA in the United States cannot of course use glass or quartz lenses to focus the electron beam For this purpose special electric and magnetic lenses are used which act upon the electron beam in a high vacuum It required ingenious research and the highest scientific exactitude to create machines which would meet the extremely demanding specifications necessary to make an actual functioning electron microscope of all the vague plans The resulting machines are such extremely delicate and complicated precision instruments and so expensive ($20 000 to $30 000) that von Borries estimated that only about 1500 were in use throughout the world in 1955

The reason that electron microscopy has as yet yielded so few returns to biology and medicine is not alone financial however The technical difficulties confronting the electron microscopist are

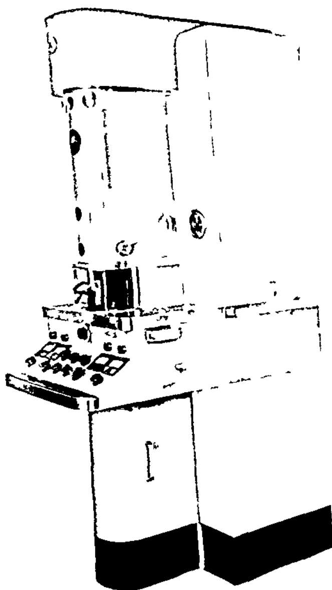


Figure 1 Exterior view of the RCA Universal Electron Microscope (Model AMU 2E) All the figures published in this volume were produced and photographed by means of a microscope of this type

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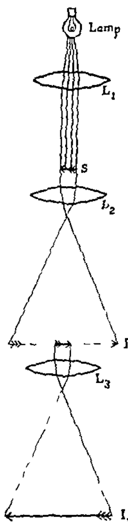
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discouraging in scope To begin with there is the question of proper specimen fixation preferably in the live state Post mortem changes occur much more quickly in the size gradient viewed through an electron microscope than in the size gradient of the conventional light microscope This is the reason that it is so hard for us to study the most vital organs such as the human heart in a state approximating life Fifteen minutes postmortem in an unfixed state are sufficient to produce the grossest changes in the ultramicroscopic structure of the heart tissues The human tissues (auricles M pectoralis) pictured in this volume were obtained unless otherwise noted at cardiac operations and the specimen was immediately fixed in 1% osmium tetroxide solution according to Palade s method (1952a) All animal tissues illus trated in this volume are likewise fixed in osmium tetroxide solu tion (0 1% up to 2%)

Despite its many virtues well known from light microscopy the osmium staining technique is certainly not the ultimate solu tion to the "staining" problems of ultramicroscopy This author like many others has been experimenting along these lines and doesn t doubt that other "stains" will bring out clearly cell struc tures which are less well seen with the present osmium technique

Another vital problem to electron microscopists is the thickness of specimen sections Electron microscopic photography exposes a photographic plate by contact of the photographic emulsion with those particles of the electron beam which manage to pass through the specimen and are not deflected and absorbed by special screens Just as the screen on a fluoroscope lights up at those places where the x rays strike it the screen of an electron microscope lights up where it is struck by the electron beam On a photographic plate substituting for the screen consequently a black area arises at those places where the electrons strike the plate instead of the illuminating screen Those parts of the specimen which do not permit passage of the electrons but scatter them to the absorbing screens appear as shadows on the observation screen and as light areas on the photographic plate Thus the picture obtained with the electron microscope like the x ray plate represents a shadow image of the denser elements of the specimen The more dense elements that there are standing



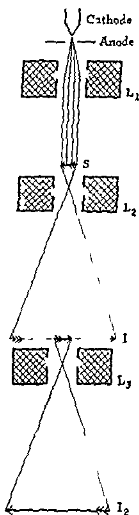


Figure 2 Comparative diagram of the beam within an electron microscope and within a light microscope This and the previous figure have been made available through the courtesy of RCA

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above or next to one another in the path of the electron beam the less usable for tissue studies is the resulting picture. It is, therefore, apparent that the thicker the sections are the more this undesirable effect will be found. For this reason, the electron microscopic study of tissue sections was until very recently highly unsatisfactory. It was, therefore thought preferable to split the specimen into dust like particles and make a suspension of these particles. An extremely thin film of this centrifuged suspension was next allowed to dry on a very fine membrane stretched over a metallic mesh and was sprayed with a molecular layer of some metal. Then the metal covered object was examined under the electron microscope.

For biology and medicine by and large such a method no matter how fruitful in its results could not be considered satisfactory. It was not histological at all; rather it was a study of artificially produced tissue fragmentation. This method of necessity completely missed the significance of sarcosomes in muscle tissue. As soon as newer methods of sectioning allowed true tissue studies, however, comparison of cardiac and skeletal muscles became possible and showed the different amount and the importance of sarcosomes clearly. Ingenious new microtomes now produce sections not in excess of 0.2 μ and often thinner than 0.1 μ. For specimens today 0.1 μ should be uniformly demanded although of course one must take into consideration here the nature of the specimen under investigation.

In order to obtain such thin sections one needs special microtomes and cutting knives (mostly glass but also diamonds). In addition the object must be embedded in a completely different way from that which was common in light microscopy. Even today much research is being done on this problem. The experiments range from attempts at freezing drying techniques (Pease and Baker 1949, Gesh 1956), to the techniques of embedding in plastic which are the ones most commonly used today (Borysko 1956).

Each one of the experimenters swears by his special method and the purely empirical decision as to which is the best remains to be decided by a comparison of the results in each technique.

In order to accustom himself to the unfamiliar *order* of magni

tude the reader is advised to consult the scale representing 1 μ (0.001 mm) for that particular magnification which is drawn on each of our pictures. Every millimeter on this scale represents a magnification of 1 000×. For example if the scale line is 20 mm in length then the magnification of the object seen in the photograph is 20 000×. At a magnification of 50 000× which is not unusual to the electron microscope a 1 mm object would be 50 meters long (about 150 feet) which is approximately the height of a large size building.

We will not go into further technical details here. These details are contained in textbooks and in various specialized publications. It should be clear after reading this chapter however why the use of electron microscopy as a research tool by medicine and biology is still in its infancy.

The following orders of magnitude which appear repeatedly in the text and illustrations are refreshed here for the reader:

1" = 1 inch = 25 mm
1 mm = 1000 μ (μ = Micron)
1μ = 10 000 Å (Å = Angstrom)
1 mm = 10 000 000 Å

II.

THE GENERAL STRUCTURE OF MUSCLE FIBER

Basically, the structure of the muscle fiber of cardiac muscle is very similar to the muscle fiber of skeletal muscle. However, the two structures differ widely in several ways.

Cardiac muscle fibers, like skeletal muscle fibers, are surrounded by a membrane which can be clearly demonstrated in the electron microscope. The structure of the membrane and its characteristics will be discussed more fully below. The membrane surrounding cardiac muscle fibers is simpler in its structure than is the sarcolem of skeletal muscle. However, in the following text, this membrane will always be referred to as the sarcolem of the cardiac muscle fiber. The sarcolem surrounds all the primitive contractile elements contained in one muscle fiber, that is, the myofibrils, which have also been referred to as the primitive fibrils. Large numbers of these myofibrils are surrounded by a single sarcolem. They represent the simplest independent contractile element of the muscle (see diagram Fig. 3). Even in electron microscope pictures of good quality these little fibrils appear as separated structures, and at the beginning of our research their appearance caused me to call them bamboo stick fibrils. Since these primitive fibrils appear as clearly separated structures within the muscle fiber, I see no reason why the heart muscle should be considered as a syncitium despite the fact that in heart muscle, in contrast to the flight muscle of insects, a parting of the myofibrils is more often seen. After all, the spinal nerves are not looked upon as a syncitium, though they arise by a joining of two nerve roots, because the individual nerve fibers are entirely separated from one another. I was not able to discover a separate membrane surrounding the individual myofibrils. This is in

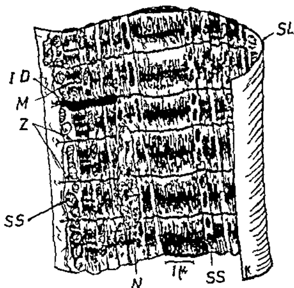


Figure 3 Schematic diagram of an uncoiled cardiac muscle fiber The individual myofibrils whose striations can be seen are connected by means of Z bands SL–sarcolem SS–sarcosome N–nucleus ID–intercalated disc M–middle line

agreement with the work of Hodge Huxley and Spiro (1954) and that of Chapmann (1954) However the outer surface of the myofibril presents an interphase in relation to the surrounding sarcoplasm which may fall into the biological category of a membrane

The only connection between the primitive fibers or *myofibrils* of the heart is in the form of lamellae or band like tiny threads which in the region of the Z-zone stretch from one myofibril to the other These tiny threads the Z bands are not identical with the Z zones of the myofibril even though when present they are always found in this region of the myofibril They are clearly demonstrable in the heart especially in ultramicroscopic teasing preparations (see Figs 4 and 25) We are not certain whether these bands are also always present in striated skeletal muscle Pease and Baker (1949) have seen them in certain striated muscles However we have been able to show with certainty that in selected skeletal muscles for example the flight muscles of

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Each of the above organelles of the cardiac muscle fiber will be discussed separately below and illustrated with pictures Each of them is found within or at the border of the heart muscle fiber Between the individual muscle fibers one also finds cells and fibers of which part belong to the connective tissue Of special significance are the blood vessels and lymphatic vessels found there As yet we have not had time to thoroughly investigate all of these structures found in the spaces between cardiac muscle fibers and therefore of the whole group mainly the ultra microscopic picture of the capillary and the intracardiac nerves will be presented here and also some interstitial cells since our experience in this direction is somewhat greater

certain insects, the Z bands are not present (see Kisch, 1955 1959a, Kisch and Philpott 1955, the reader is also referred to Figures 21, 27, and 27a) More will be said in later chapters about the *Z bands Z zones and the zones surrounding Z zones* (PZ) Our latest investigations seem to prove the absence of the Z bands, but presence of Z zones also in myofibrils of the conductive system of the heart (see below)

A further, regular component of cardiac muscle is the nucleus with its enclosed nucleoli Of great significance also are the small bodies, already seen early in the last century by Bowman, Henle and Koelliker, which until 1950 received no attention from either physiologists or cardiologists, though at the turn of the century such prominent anatomists as G Retzius and Holmgren had shown interest in these organelles Despite the indifference shown them these little bodies are vital components of the heart muscle (B Kisch 1952a 1952b 1957g), they are the sarcosomes The name sarcosome was applied to the little bodies in 1890 by G Retzius who observed them mainly in the flight muscles of dragonflies They are also present in varying lesser numbers in skeletal muscles and their abundance depends on the type of the muscle in question (see Kisch 1951 1956f) They are found in countless numbers in the heart muscles of all animals which we have so far investigated (mammals man fish, frog)

Finally, one other structure must be mentioned It is a very fine membrane which has been found occasionally within the heart muscle fiber and whose physiological significance is not yet understood Some of these structures may perhaps be part of the endoplasmic reticulum which will be discussed later or belong to that system which earlier histologists called Ergastoplasm

We would also like to mention smallest droplets present within the muscle fiber in the cytoplasm around the myofibrils They are apparently not part of the endoplasmic reticulum Their diameter is about 300–1000 Å They serve probably in the exchange of material between muscle fiber and the intercellular space much as do similar droplets in the capillary wall and in *nerve axons* (*see* Fig 78) I have called this important procedure droplet transfer or dropletdialysis in contradistinction to the well known molecular dialysis (Kisch 1960)



Figure 4 Muscle fibers from the auricle of an albino mouse SL—sarcolem It should be noted that the Z bands connect the individual myofibrils and are connected with the sarcolem at the place where it is indented The numerous sarcosomes and microsomes of varying sizes and form are all found within the muscle fiber partly between the myofibrils partly between the sarcolem and the myofibrils and partly in proximity to the nucleus (N) Arrows indicate sarcosomes near the nucleus 12 000X

III.

THE SARCOLEM

The ultramicroscopic structure of the sarcolem of the skeletal muscle of insects and mammals has been repeatedly described and pictured in recent years (Draper and Hodge, 1949, B Kisch, 1951, 1955c, Bennet and Porter 1953 H Ruska, 1954 etc)

The muscle fibers of the hearts of all vertebrates investigated thus far, including humans, show a thin encompassing membrane which in the electron microscope appears as a double boundary line approximately 200 Å to 300 Å in thickness The membrane appears free of all connective tissue and therefore it is more delicate than the sarcolem of skeletal muscle The membrane has been described by Draper and Hodge (1949) B Kisch (1951 1957g) and others

In some hearts as we were able to show groups of thin muscle fibers together with their sarcolems are grouped into larger bundles of fibers by means of a further membrane extending from cells having definite nuclei Thus we could differentiate a true sarcolem as described above from this other more variable membrane (B Kisch 1954 1957g) This other membrane is not a simple sarcolem Rather it has the appearance of cells lying between the muscle fibers having clearly visible nuclei and which enclose by means of their protoplasm a group of muscle fibers together with their sarcolems (see Figs 5 and 6) We shall return to this subject in a later chapter The designation sarcolem of the second order should not be construed to mean that these membranes are of the same sort or of the same origin as the true sarcolems of the separate cardiac muscle fibers which have no nuclei of their own Rather these second order sarcolems are made up of cells whose cytoplasm encloses groups of muscle fibers in pipe like fashion (Fig 6) I would further hesitate to



Figure 6 Cross section through muscle fibers of the auricle of an albino mouse Here as in the fish heart (Figure 5) one sees muscle fibers with a true sarcolem (SL) bound together by a sarcolem of the second order" the latter being made up of the cytoplasm of a neighboring interstitial cell which surrounds the muscle fibers in the manner of a pipe Several different types of plasmasomes are seen within its cytoplasm 12.000×



Figure 5 From the ventricle of a pipefish A number of cross sectioned muscle fibers whose true sarcolem (SL 1) is recognizable are enclosed membrane like by a second order sarcolem (SL-2) This second order sarcolem is made up of wing shaped extensions of cytoplasm of cells whose nuclei (N) are easily recognized Within the muscle fiber one can see the ribbon shaped perpendicularly sectioned myofibrils (MF) and the smaller rounder sarcosomes (SS) 7 000×

IV

THE MINUTE MUSCLE FIBRILS (MYOFIBRIL, MUSKELFIBRILLE, MYOFIBRILLE)

The muscle fibril like the muscle fiber has a double striation axial and transverse The transverse striation (running parallel to the transverse diameter) is represented by the bands and zones known to us from light microscopy which are responsible for all of the cross striation of striated musculature The axial striations which run parallel to the long axis of the muscle fibril are by virtue of the structure of the fibril made up of the finest of structures the arch fibers or protofibrils In this respect no major difference can be demonstrated between the myofibrils of skeletal musculature and those of cardiac muscle As our pictures illustrate both show basically the same structure

The width of the individual myofibrils varies considerably in the heart of different animals (see Fig 3) For the rabbits ventricle the average width of myofibrils is 0 86μ (range 0 2μ–2 2μ) For the cow it is 1 82μ (range 0 4μ–5 2μ) For the human ventricle the value is approximately between these two but there are no values available as yet for the normal human heart

designate this structure as being similar to the perimysium (Bruno Kisch 1954, 1957g, recently, Fawcett and Selby, 1958)

It is typical of the first order or true sarcolem of the individual muscle fibers both in the ventricle and in the auricle that when it is cut lengthwise, it usually has the appearance of a garland (see Figs 4, and 23) The indentations of this garland are often visibly attached to the Z bands, which bind together the myofibrils Frequently, it is very easy to recognize this attachment (see Figs 4 23 and 24) We shall discuss this more fully later This appearance had already been described in regard to skeletal muscle by light mocroscopists and electron microscopists have since confirmed the observation in part (Pease and Baker, 1949) It definitely occurs in heart muscle (Kisch 1951 1957g and more recently Poche and Lindner 1955 Lindner 1957) The indentations are always found in the region of the Z band even when the attachment to the indentation of the sarcolem cannot be clearly demonstrated All this is also true of the human heart (see Figures 23 and 24) In the pockets of the sarcolem created by the above described outpouchings one often finds one or more sarcosomes lodged between the sarcolem and the myofibrils (B Kisch 1951 1957g) In auricular muscle fibers one frequently finds numerous sarcosomes in these sarcolem pockets (B Kisch, 1956a, b and f) Less frequently the same is true in muscle fibers from the ventricle (see Figs 4 and 31)

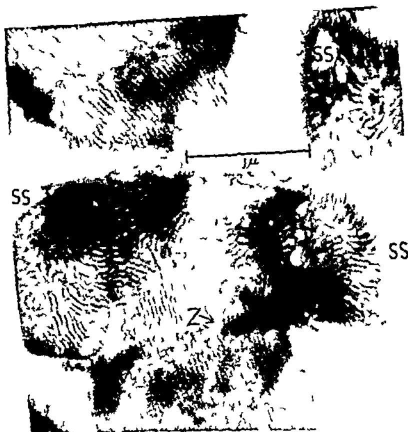


Figure 7 Sarcosomes and myofibrils from the flight muscle of a wasp In this figure the protofibrils and the lamellar structure of the interior of the sarcosomes is clearly demonstrated as is the fact that the protofibrils traverse the Z zone The distance between the center of one protofibril and the center of the neighboring one is approximately 300 to 350 Angstroms Notice also the little vacuoles in the sarcosomes (SS) 34 000× (From Kisch and Philpott, 1955)

being held together only in the region of the Z band A different picture results if a muscle fibril instead of being sectioned along the longitudinal axis is sectioned obliquely or transversely The protofibrils are then seen as parallel lines or frequently as parallel dotted lines in which each dot represents a protofibril which has been cut across obliquely In cross section the protofibrils appear as regular dots packed in a hexagonal pattern The distance be

V

THE PROTOFIBRILS (MYOFILAMENT, PROTOFIBRILLEN, URFASERCHEN)

In longitudinal sections of a muscle fibril, one can recognize the protofibrils as striations running parallel to the long axis of the fibril. The distance between the center of two of these striations is approximately 250 to 350 Å and the diameter of a protofibril is approximately half of this distance. Bennet and Porter (1953) have stated that the diameter of a protofibril of the breast muscle of a chicken is approximately *150 Å, while Hodge (1955) give* the same diameter in the muscles of insects as approximately 120 Å and in mammals and amphibians as 100–130 Å (Hodge Huxley and Spiro 1954). In my own investigations I found on the average the following measurements for the protofibrils of the heart 150 Å in the heart of a bat (1954) 200 Å in the heart of the guinea pig (1955), approximately 150 Å in the ventricular *musculature of the human heart (see Figs 7, 8 9 10), and ap*proximately 215 Å in the human pectoralis muscle. Philpott (1954) states that the diameter of the protofibril of the flight muscle of the wasp is 220 ± 15 Å while Chapman (1954) gives the diameter in the same tissue as 100 Å. The diameter of the protofibrils in the heart of mammals has been found to be 100–150 Å by Moore and Ruska (1957) and that of the rat 110 Å (Porter and Palade 1957). In the wing muscle of the meat fly I recently found the center to center distance of the individual protofibrils circa 350 Å. That would mean a protofibril diameter of circa 170 Å. Occasionally if the specimen has not been properly fixed and therefore, *the tissue structures have split apart one finds on* examining these ultra microscopic teasing preparation that the fibrils have been split up into bundles of protofibrils the bundles



Figure 9 Cross sectioned myofibril of the same human heart in bigger enlargement From Kisch Cavusoglu and Marangoni (1959) 70 000×

the mammalian heart the distance is about 300 Å (see Figs 8 9 and 10) I have observed hexagonal packing in the cardiac muscle of the human auricle as well Hodge Huxley and Spiro (1954) and Hodge (1956) also mention this particular type of organization of the protofibrils of the muscle fibers in the heart I found the distance between two protofibrils as measured from center to center in the guinea pig and in the rat heart muscle sectioned lengthwise to be approximately 300 to 400 Å and in recent investigations I found the center to center distance of the protofibrils in cross sectioned myofibrils in the human heart circa 300 to 340 Å and in the flight muscle of the bumble bee circa 300 to 350 Å (see Figs 7 8 and 9)

The hexagonal packing of the protofibrils of the muscle fibril reminds one strongly of the latticed structure of crystals I have



Figure 8 Cross section of a myofibril of the human ventricle showing the hexagonal packing of the cross sectioned protofibrils The big dark bodies are sarcosomes 40 000×

tween such dots is as follows in insect muscles approximately 300 Å (Chapmann 1954 Hodge 1955) in mammals and amphibians 200 to 300 A (Hodge Huxley Spiro 1954) in guinea pig heart approximately 300 Å (according to my own findings) Moore and Ruska (1957) found it to be 280 to 330 Å in the heart of mammals The above mentioned hexagonal packing of the protofibrils which we show here in our pictures of heart muscle has already been repeatedly shown in the striated skeletal muscles of both mammals and insects (first in 1950 by Morgan and his co workers and more recently with a series of excellent pictures in insect muscles by Hodge (1955 1956)) In specimens of insect muscles Hodge (1955) estimates the distance between proto fibrils to be 600 Å As previously mentioned in our pictures of

Philpott (1955) in the flight muscle of the bumble bee and like wise by Hodge in insect muscle (1955). The protofibrils appear as thickened and darker in the M and in the Z zone (see Fig 7) in mammals as well as in the human heart. In vertebrate muscula ture a similar occurrence has been shown in metal shaded preparations

TABLE I
DIAMETER OF MYOFILAMENT

Specimen	Animal	Å	Author
1 Wing muscle	Insect	120	Hodge 1955
2 Wing muscle	Meat fly	170	Bruno Kisch 1954 1959
3 Flight muscle	Wasp	20	Philpott 1954
4 Flight muscle	Wasp	100	Chapman 1954
5 Breast muscle	Chicken	150	Bennet Porter 1953
6 Muscle	Mammals Amphibia	100–130	Hodge Huxley Spiro 1954
7 Pectoralis muscle	Man	215	Bruno Kisch 1954 1959
8 Heart	Bat	150	Bruno Kisch 1954 1959
9 Heart	Guinea pig	up to 200	Bruno Kisch 1954 1959
10 Heart	Man	150	Bruno Kisch 1954 1959
11 Heart	Mammals	100–150	Moore and Ruska 1957
12 Heart	Rat	110	Porter and Palade 1957
13 Heart	Do Guinea pig Frog	120	Lindner 1957
14 Heart	Cow	ca 150	Bruno Kisch 1959

TABLE II
CENTER TO CENTER DISTANCE OF MYOFILAMENT

Specimen	Animal	Å	Author
1 Wing muscle	Insect		Chapman 1954
		300	Hodge 1955
2 Muscle	Insect	600	Hodge 1955
3 Wing muscle	Bumble bee	300–350	Bruno Kisch
4 Muscle	Mammal Amphibia	200–300	Hodge Huxley Spiro 1948
5 Heart	Guinea pig	300	Bruno Kisch 1954
6 Heart	Rabbit	300	Bruno Kisch
7 Heart	Rat	300–400	Bruno Kisch
8 Heart	Man	300–340	Bruno Kisch
9 Heart	Mammal	250–300	Moore and Ruska, 1957
10 Heart	Cow	350–400	Bruno Kisch 1959
11 Wing muscle	Honey bee	300	Bruno Kisch

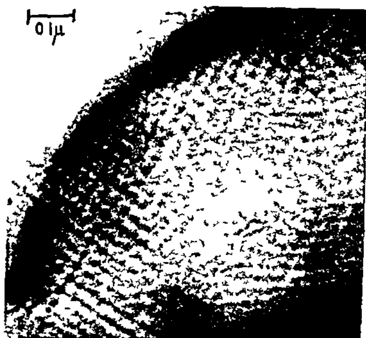


Figure 10 Cross section of myofibril of the wing muscle of a bumble bee showing the protofibrils in hexagonal packing 100 000×

repeatedly pointed out the fact (1951a 1954b etc) that the requirements demanded of a crystal—namely homogeneity of the tiniest similar component parts among each other double refraction of a light beam, and the existence of an optical axis—can also be applied exactly to skeletal muscle and cardiac muscle and that furthermore the physical properties of muscles are such that one should consider including them under the classification of Otto Lehmanns liquid crystals Further studies are still urgently needed here

It would seem that the protofibrils pass without interruption through the various zones of striation of cardiac muscle (Hodge 1956) as can be seen especially well in ultramicroscopic teasing preparations This has been shown by various authors to be the case in the skeletal muscles of vertebrates and in the flight muscles of insects (see Hall Jakus and Schmitt 1946 Rozsa Szent Gyorgyi and Wyckoff 1950 Bennet and Porter 1953 Chapmann 1954 Hodge, 1956) A clear example of the fact that the proto fibrils also pass through the Z zone was pointed out by Kisch and

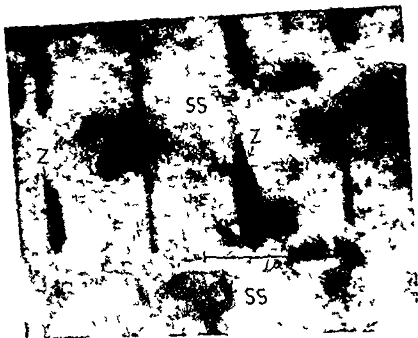


Figure 11 Myofibrils from the ventricle of a bat Sarcosomes are visible between the myofibrils (SS) The ultramicroscopic cross striation is clearly visible occurring at a spacing of approximately 300 to 350 A 40 000×

It seems therefore that ultramicroscopic rhythmicity of the muscle fibril occurs as universally in the cardiac muscle of vertebrates as according to Hodge (1949 1956) it does in their skeletal muscles

The measurements of ultramicroscopic and microscopic structures as given here and in the following chapter are summarized in Tables I and II They depend unquestionably not only on the functional stage of the tissue at the moment of fixating but also on the different technical procedures to which the tissue is submitted during its preparation for electron microscopic investigation This may well explain the differences in the findings of different investigators The figures represent fairly well however the average magnitude of the measured values and in this regard the results of different authors are yielding corresponding results very well

VI

ULTRAMICROSCOPIC TRANSVERSE STRIATIONS OF MUSCLE FIBERS

In cardiac muscle, as in skeletal muscle, the familiar striations of the cross striated muscle fiber are determined by the cross striations of the myofibrils. Two types of cross striation must be differentiated: those of ultramicroscopic and those of microscopic order of magnitude.

In the ultramicroscopic order of magnitude we have the transverse rhythm of the protofibrils first described by Hall, Jakus and Schmitt (1946) as spaced approximately 400Å apart in the fibrils of dessicated skeletal muscle. According to investigations by Draper and Hodge (1949), this ultramicroscopic cross striation is a generalized occurrence seen in metal shaded preparations of dessicated skeletal muscle as well as in ultra thin sections of skeletal muscle. According to these authors the distance between the ultramicroscopic cross striations is dependent upon the state of contraction of the myofibril. The transverse periodicity of striated muscles seems to affect more than the protofibrils because, according to Hodge, it also involves the tiny bridges which bind the protofibrils together (1956). In the striated muscle of man (psoas and pectoralis) I have been able (1957g Figs. 7 and 8) to demonstrate ultramicroscopic rhythmicity in bands separated by approximately 250–300 Å. In cardiac muscle I first observed these striations in the auricle of the bat (1954) where their spacing was approximately 250–300 Å. In the guinea pig I found the striations (1955a, b) in the auricular and ventricular musculature (300–350 Å) and I likewise found them in as yet unpublished studies on the auricle and ventricle of the mouse. Recently this rhythmicity of the heart muscle fibril (250–300 Å) has also been confirmed by Hodge (1956).

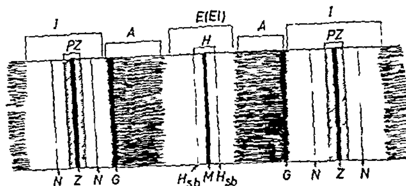


Figure 12 Diagram representing in schematic fashion the zones of a sarcomere Most of these zones are visible in various portions of Figure 13

Z zone also appears as the darkest of the striations It would seem that the muscle fibril is especially brittle at this point In cases where some force is applied to the tissue under examination one most frequently sees tears or separations of the myofibril in the region of the Z-zone In such an event as illustrated in Figures 14 15 and 16 Z either remains attached to one piece of the broken fibril or else it becomes detached from both pieces thereby actually appearing as a disk (see Fig 16 at the arrow) however it does not appear as a disk crossing the muscle fiber but only the myofibril

Approximately midway between two Z-bands one usually finds a second sharply delineated line which light microscopists have referred to as the M zone (M=*Mittelscheibe*) even though M like the *I* is neither a true band nor a membrane in the usual sense of the word As a rule M is thinner than Z

Two lighter zones approximately equal in width are found one on either side of the Z-zone These light zones have been given the name I ("Isotropic Zone") Within the I zone one finds on either side of Z one rarely two thin lines designated as N by their discoverer Engelman (N = *Nebenscheibe*) Frequently one finds a thin dark zone but lighter than *I* directly adjacent to Z and occasionally extending to the immediate border of N This zone was designated as PZ (Para Z-band zone) by Kisch and Philpott (1954)

On either side of M one finds a light zone H named after its

VII

THE MICROSCOPIC CROSS STRIATION

The literature concerning the discovery, via the light microscope, of the striation of skeletal muscle is very extensive (see the summations by Cohnheim, 1865, Krause 1868 G Retzius 1881 Melland 1885, Rollet, 1885 Koelicker, 1888 McDougall 1897, Meigs 1908, Heidenhain 1911, Haggquist, 1931 Barer 1948 Jordan 1920 1934, 1955 and many others). The nomenclature has not at all times been equally standardized. The diagram in Figure 12 is meant to show the manner in which we apply the *usual nomenclature to cardiac muscle. As regards the myofibrils* it should be stated at the outset that we have thus far been unable to find significant differences microscopically or ultramicroscopically between those of cardiac and those of skeletal muscle as to their longitudinal and transverse striations with the exception of the Z bands which will be discussed below. This fact was already apparent in the studies made upon dessicated cardiac muscle (Beams Evans Janney and Baker 1949).

The repetitive order of the various striations produces a segmentation of the muscle fibril, and therefore also of the muscular fiber dividing all into a succession of identical minute units the *so called sarcomeres*. In the older literature the sarcomeres were also referred to as little muscle boxes (*Muskelkastchen*). The boundary line of each sarcomere is the densest of the dark cross striations. *This line has been well known since the time of Dobie* (1849) and Krause (1868) and designated as the *Z* zone (the *Z* stands for Krause's term *Zwischenscheibe*). The word *Scheibe* means disk. However none of the zones of the striated muscle or of the heart muscle is really a disk crossing the muscle fiber.

In the electron microscopic picture (with osmium stain) the

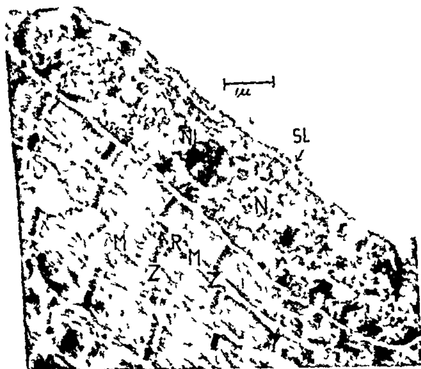


Figure 14 Muscle fibrils from the human pectoralis muscle Directly beneath the sarcolem (SL) there is a nucleus (N) containing a nuclear body (nucleolus NL) The myofibrils clearly demonstrate the Z and M bands and also the longitudinal striations determined by the protofibrils R = endo plasmic reticulum 13 000×

discoverer Hensen Within this light zone and parallel to M on either side of the dark zone one can recognize one occasionally even two stripes These are designated as Hsb (H sub-bands) by Hall Jakus and Schmitt (1946) and Draper and Hodge (1949) These same stripes were designated by Beams and his co workers (1949) as X stripes Between H and I one finds a broad dark zone Zone A (anisotropic zone) which at its boundary with I shows occasionally a distinct darker stripe which I have designated as G (*Grenzstreifen*) (see Figs 12 17 18)

This maze of zones at first somewhat confusing but regular repetitive and probably characteristic occurring within the



Figure 13 Myofibrils from the ventricle of a guinea pig. The individual zones can be clearly recognized and are labeled with appropriate letters. The dark stripes surrounding the Z zone represent the para Z band zone (PZ). M = middle line SS = sarcosomes 32 000×



Figure 16 Fibrils of human pectoralis muscle At points indicated by arrows the Z zone is separated from both adjacent sarcomeres At the upper end of the picture the fibers have been torn in very close proximity to the missing Z zone M = middle line 29 000×



Figure 15 Fibrils of human pectoralis muscle torn directly at the Z zone 20 000×

sarcomeres is found in cardiac muscle both of the auricle and of the ventricle (Bruno Kisch since 1951 Lindner 1957 Moore and Ruska 1957) and also in skeletal muscle The biologic significance of these zones is not yet known but hints at rhythmic biochemical differences within the sarcomere

Figure 12 summarizes all of the above in a simplified fashion

As far back as 1933 Jordan observed a widening of the H zone upon stretching of skeletal muscle This was confirmed electron microscopically by Pease and Baker (1949) D Philpott and A Szent Gyorgyi (1953) linked this zone which upon expansion of the muscle is clearly visible between H and A with the elastic elements of the muscle fibril and called it Zone E (A Szent

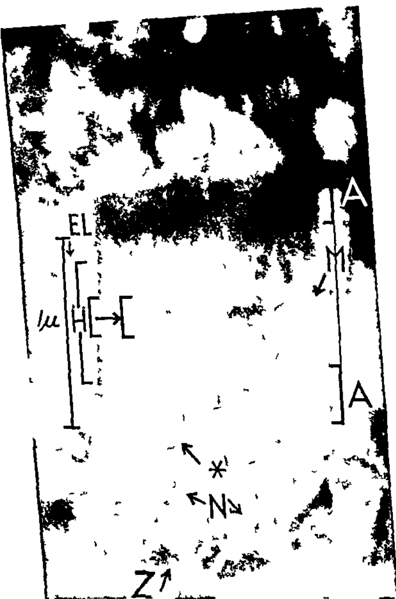


Figure 18 Myofibrils of the same psoas muscle (newborn) illustrating a different state of muscular tension Between the two A zones there is at either end a broad light zone (El) Again here the dark G band (↑) is visible running parallel to the N band and bounding the A zone 50 000×

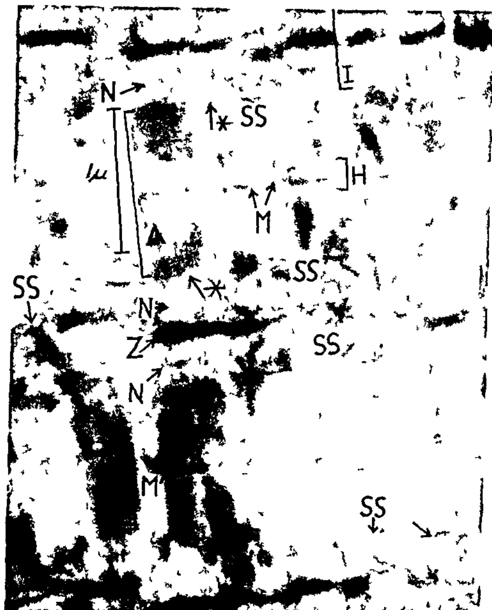


Figure 17 Muscle fibrils of human psoas muscle showing all the typical zones One can clearly recognize Zone N running parallel to the Z band and a dark stripe marked with a star where Zone A bounds on Zone I This boundary line has been designated as G (*Grengzstreifen*) In the region of the Z band Z band sarcosomes are visible singly or in pairs (SS) 32 000×

(B Kisch 1951) and can be best demonstrated in ultramicroscopic teasing preparation Pease and Baker (1949) also claim the existence of such Z bands in skeletal muscle It remains to be proven however that these Z bands of skeletal muscle are as constant as are those of cardiac muscle Although the insect s flight muscles show the Z zone very clearly as in Figures 21, 22 27 27a our own research has failed to discover distinct Z bands connecting the individual myofibrils in the flight muscles of the horse fly meat fly bee bumble bee wasp and hornet (Kisch 1955c) Chapmann s (1954) photographs of the flight muscles of various insects agree with these observations It seems that the same is true for the myofibrils of the Purkinje Fibers (see below) in the mammalian heart

The Z bands and fibrils of cardiac muscle produce a weave pattern in which the Z bands are connected on either side with the sarcolem I have therefore spoken of the structure of the heart muscle (myocardium) fiber as being similar to a warp and woof pattern That these Z bands cannot be demonstrated in every longitudinal section of cardiac muscle is secondary in importance to the fact that they are frequently very noticeably demonstrable (see Fig 4) This is equally true of the human *heart as it is* of the hearts of all vertebrates investigated thus far *I have* been emphasizing this point repeatedly since 1950 and *recently it has* been confirmed in the rat heart by Hodge Huxley and Spiro (1954) *There are* several reasons why the *Z bands* are frequently *not visible* The *most* important reason seems to be that these bands are in reality bands and not plates Therefore with our ultra thin *sections* we by no means always manage to catch the *bands since* they are neither always found lying in the direction of sectioning *nor apparently* are all the bands always found to be running *parallel in the* same direction The connection of the Z-bands *to the sarcolem* has been found again and again (Kisch 1951 Van *Breemen* 1953 Hodge Huxley Spiro 1954) One result *of* this attachment *is that* the sarcolem of cardiac muscle fibers when *sectioned longitudinally* often appears as a serrated edge (*garland shape*) The indentations of the garland are as one can clearly see from our illustrations attached to the Z-band The physiological result of such in

Gyorgyi 1953) These same authors also connected the changing thickness of Zone I with the elastic elements of the muscle fiber and with their tone (1953) Our Figures 17 and 18 show varying degrees of stretching of the fibrils of the same human psoas muscle

Unfortunately Rollet, in 1885, already used the designation E to signify a different striation found in the neighborhood of the Z-band It would perhaps therefore, be better to designate the new zone, linked with the elastic elements of the muscle as El, rather than as E

Of all the above mentioned zones and bands only the Z stripe, the boundary of the sarcomere, can be relied upon as a constant and unchanging phenomenon In good photographs, M also is usually though by no means always present Whether it is justified in the absence of all other distinguishable zones to designate a uniformly dark region stretching from one Z to another as A, is I think, open to much questioning In summary it can be said that Z is always present in electron microscopic pictures of the muscle fibril (skeletal and cardiac muscle), while the ability to demonstrate all the other zones of the sarcomere is dependent upon the condition of the muscle at the moment of fixation Tension or relaxation activity or inactivity of the tissue being examined, all strongly influence the possibility to demonstrate any of the zones except Z In the course of embryological development it would appear that Z is the first demonstrable stripe (Van Breemen 1952) As far as we know, Z is present not only in the muscles of vertebrates and insects but it is also invariably present in the muscles of the crustacea (Farrant and Mercer 1952) The physiological significance of the variability of the striations in striated muscle as regards the function of the striations has been the subject of many studies and much speculation for over a century although as yet the problem has not been satisfactorily solved

As previously mentioned *the ultramicroscopic structure of the cardiac muscle fibril is essentially identical with that of the skeletal muscle fibril of the same animal This is equally true of the auricle and of the ventricle* Cardiac muscle however has a constant finding of thin bands in the region of the Z zone which connect the myofibrils of the myocardium with one another

arrangement may well be that for example in the heart where due to the sarcosomes the myofibrils are but loosely packed together the structural order may still be maintained during contraction much as Pease and Baker (1949) suspected to be the case for skeletal muscle I have emphasized this for cardiac muscle repeatedly (1952a b 1952c) Szent Gyorgyi has also recently championed this idea (1956)

The fact that the Z zone the L band and when present the PZ zone are always found approximately in the center of the light I zone leads one to speculate that the L band—and also the Z zone—may have something to do with the origin of the I zone (Kisch 1951 1957g) It must be said however that nothing certain is known about this It would also be pure conjecture to argue that the relation of the Z zone to the N zones or that of the M zone to the Hsb zones reminds one of the formation of the layers which bear the name of Liesegang (*Liesegangsche Schichtung*)

Commonly in pictures of insect wings and more rarely also in pictures of cardiac muscle one can see that the protofibrils appear to pass through both the Z and the M zone and not just through the latter In this region the protofibrils are thickened and the substance between them is darker than is the case outside of Zone Z (Kisch and Philpott 1955 Lindner 1957) It is not yet possible to decide whether we are dealing here with concentrations and encrustations of minerals as Hodge (1949) has postulated

The great variation in appearance of myofibrils even within the same muscle is illustrated by our photographs of various myofibrils of the same human psoas muscle According to Philpott and Szent Gyorgyi (1953) the variable expansion of the EI zone is proof of the fact that different myofibrils of the same section of muscle were in different degrees of tension at the time of fixation (see Figs 17 and 18)

It is well known that Zone I and Zone H can both disappear completely when the muscle is in its contracted state Sometimes M also disappears and then only the bounding Z zones remain Zone H will occasionally appear with sharp and clear borders while M is missing (see Fig 19 from the heart of a pipe fish)



Figure 19 Ventricle of a pipefish Myofibrils and sarcosomes The M line is missing from the clearly recognizable H zone 25 000×

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VIII

PSEUDO STRIATION OF THE MYOFIBRILS

A J Hodge and his co workers (Hodge, Huxley and Spiro 1954, Hodge, 1955) were perhaps the first to point out the fact that in addition to the well known cross striations of muscle fibers electron microscopic pictures may contain striations which are artifacts and have nothing to do with the organic structure of the myofibril They designated this form of striation as pseudo striation

Hodge (1954) explains these pseudo striations by postulating that a muscle fibril has been cut at an angle by the ultra thin sectioning technique whereby its regularly ordered protofibrils have also been cut at an angle row upon row Thus the section cuts the protofibrils arranged in rows in a step wise fashion which produces a picture of a succession of light and dark bands Our schematic drawing (Fig 20) attempts to show this If the *pseudo striations take the form of concentric arcs these same* authors (1954) claim that a twisting of the material in the course of fixation and embedding is to blame

Our Figures 21 and 22 show cases of these pseudo striations I do not believe that they could result from either an ultra microscopic crumbling of the specimen or from regular variation in the thickness of the section attributable to vibration of either the microtome or the specimen in the course of sectioning In the former case the periodicity of light and dark seen occa*sionally would have to affect the whole section sarcosomes* and muscle fibrils alike This however is not so In the latter case it would be hard to understand how the pseudo striations could achieve an order of concentric arcs

I believe that the pseudo striations could be the result of

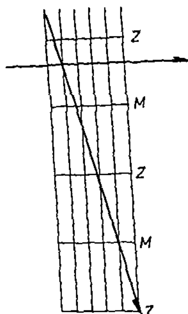


Figure 20 Diagrammatic representation of a cross section and diagonal section through the protofibril of a muscle fiber

several widely different phenomena of which only two will be discussed here

First a parallel arrangement of bands can doubtless result as Hodge has emphasized (1954 1955) from oblique sectioning through muscle fibrils wherein the section cuts across adjacent rows of protofibrils in step wise fashion and the interspace between the rows appears electron optically less dense than do the protofibrils themselves This is illustrated in our Figures 21 and 22

Second in isolated cases we may be dealing with a rhythmic succession of zones more or less densely stained by the osmium or other stain In this connection we should think primarily of the cases showing wide concentric arcs Such an appearance would be particularly likely in sections where the dark pseudo striations make recognition of the cross section protofibrils impossible provided that the penetrating osmium solution is producing the phenomenon of Liesegang layers in these sections



Figure 21 Flight muscle of a meat fly Note the pseudo striations These are explained in part by the diagonal plane of sectioning in part by a distortion of the fibrils through the plastic embedding material and in part there is no explanation for them Note also the cytoplasmic matrix in which the sarcosomes (SS) are embedded 13 000×

Several things are in favor of such an interpretation For one it allows for the fact that the striations may occasionally run almost parallel to the longitudinal axis of the fibril More important however is the fact that one occasionally sees as in our Figure 21 that the concentric arcs become more and more widely separated from one another the further out they are from the imaginary center This exactly is a typical property of the Liesegang layers when they occur by precipitation of a suitable



Figure 22 Flight muscle of a meat fly Sarcosomes are visible between the myofibrils Note the plastic deformity of the sarcosomes and two intersecting groups of pseudo-striations indicated by an arrow 20 000×

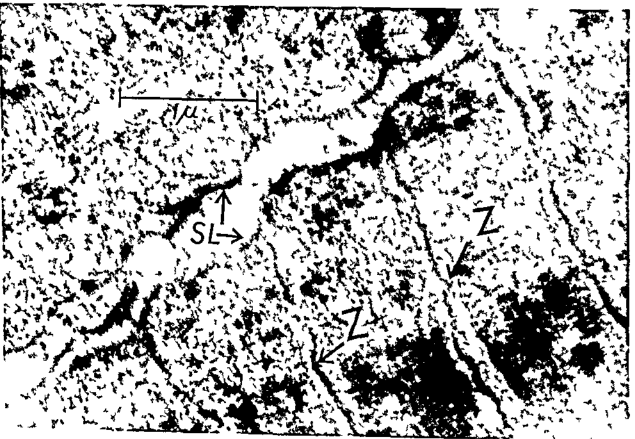


Figure 23 Myofibrils of the human auricle Some of the Z bands are clearly connected with the sarcolem 32 000×

substance infiltrating into a colloid medium A similar effect could be achieved however if an obliquely sectioned myofibril were bent within the embedding material The internal tension thus built up could lead to a fan like spreading of the protofibrils within the myofibril Ordinarily, with the usual undisturbed arrangement of the myofibrils the protofibrils would have run parallel to one another The result in both these instances would be the same In the postulated oblique section the formations of protofibrils would be increasingly separated from one another as the distance from the center of tension increased

One should also note the not easily understandable crisscrossing of various pseudo striations within the same sarcomere as illustrated in our Figure 22 (arrow)

Finally we should also point out that the pictures of pseudostriations both Hodges and Chapmann s (1954) and ours are all seen in the flight muscles of insects I have never observed the above described concentric arch formation of pseudo-striations as clearly in any other muscles On the other hand I long ago observed and reported on the parallel striations which may result from the transverse sectioning of a myofibril of the heart (1954a)

IX

THE SARCOSOMES AND THE SARCOSOME THEORY OF THE FUNCTION OF THE HEART

Since the significance of sarcosomes to date still remains largely unrecognized among physiologists, pathologists, and cardiologists, despite the fact that they have been proved to be vital components of the heart muscle fiber (B. Kisch 1951, 1952a, 1957g), it is necessary here to discuss these structures in some detail.

A. NOMENCLATURE

The story of the discovery and investigation of sarcosomes, especially the much studied sarcosomes of the flight muscles of insects, will not be gone into here, as we thoroughly covered it in 1952 (a). In order to avoid misunderstandings, however, the nomenclature requires clarification. Sarcosomes were so named in the year 1890 by the outstanding Swedish anatomist Gustav Retzius. They are the tiny so-called interstitial granules of the protoplasm, the largest of which are visible even through the light microscope. They have been known to light microscopists since the year 1840 (Bowman 1840, Henle 1841, Koelliker 1851, and many others). As these granules are found in the protoplasm of muscles, G. Retzius called them "sarcoplasmasomes" and abbreviated this name to the linguistically handier word *sarcosome*. For historical reasons and also out of respect for an outstanding scholar, we are strongly inclined to continue the usage of this name, and indeed there is no good reason for replacing this term by one which would probably be less correct.

The term mitochondria, frequently used to designate the tiny

inclusion bodies in the protoplasm can be literally translated as ribbon shaped structures" Especially in insect muscles however most of the sarcosomes are anything but ribbon shaped Since the sarcosomes of insect muscle are exceptionally large it is very simple to convince oneself of this fact with a light microscope

I was able to show the high degree of plasticity of the material from which the sarcosomes are built (1957g) Therefore sarcosomes that are spherical in free suspension may be squeezed into any shape by the pressure of neighboring organelles (see Fig 22) The same is true for the majority of sarcosomes of cardiac muscle Philologically speaking therefore it is not justified to designate a spherical or an egg shaped object as a ribbon

These then are the reasons why the sarcosomes of cardiac muscle must not be designated as mitochondria though occasional *ones* might be seen to possess a mitochondrial shape Therefore as has been our custom the little bodies composed of a sub structure which will be discussed in the following chapter will be designated as sarcosomes whenever they occur in muscle fibers Where the little bodies are found in the protoplasm of non muscular structures (for example in the wall of the capillary) they will be designated as plasmasomes or if they really show a ribbon shaped appearance even as mitochondria

B LOCATION STRUCTURE AND FUNCTION OF THE SARCOSOME

The extensive literature concerning the light microscopy of sarcosome (summarized by Kisch 1952a) was until the end of the Nineteenth Century concerned mainly with proving the existence of these bodies Even this did not go altogether unchallenged although the majority of histologists became convinced of the existence of sarcosomes at the end of the Nineteenth Century thanks mainly to the outstanding investigations of G Retzius (1890) Holmgren (1907–1910) Meigs (1908) and others The exact location of the sarcosomes within the muscle their structure and their function however remained in doubt or totally unelucidated

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Figure 25 Left ventricle of an albino *mouse* (*Ultramicroscopic teasing preparation.*) This poorly fixed preparation illustrates myofibrils and the sarcosomes which lie in *between them.* The well preserved *I bands* (Z) aligned with the myofibrils in warp and woof pattern are especially noteworthy TR = *ring shaped structure* 13 000×

tween individual myofibrils or between myofibrils and the sarcolem and can be aggregated in large quantities in the pockets of the sarcolem as we for example have found to be the case commonly in auricular muscle and in the striated muscle of the diaphragm (1955a 1958a f) Figures 24 25 26 illustrate an aggregation of sarcosomes in cardiac muscle

In muscle fibers especially rich in sarcosomes such as those of



Figure 24 Human auricle The sarcolem (SL) presenting a double line is seen in several places to be connected with the Z band of the neighboring myofibril Sarcosomes are seen to lie between the myofibrils 18 000×

Where are the Sarcosomes in the Heart?

After years of personal investigations on this subject we can state that the sarcosomes are always found within the muscle fiber and never within the myofibril (B Kisch 1951) or between different muscle fibers except for artifacts They are found be

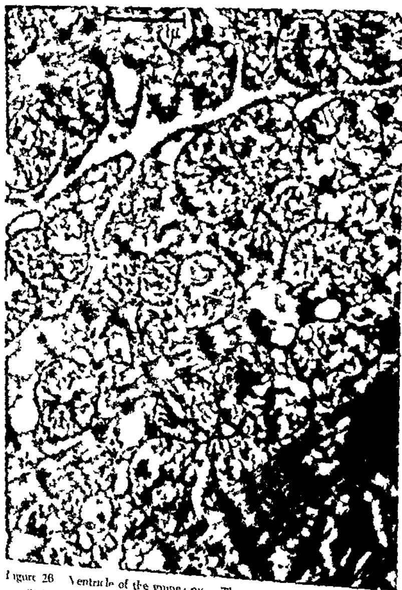


Figure 26 Ventricle of the guinea pig The mass of sarcosomes is in this picture much bigger than that of the scanty myofibrils 22 000×

the heart or the diaphragm the sarcosomes are often found lying in long rows between the myofibrils There they are tightly packed together and since their substance is evidently very plastic in nature, they often appear in the electron microscopic picture, to be impinging upon and flattening one another when ever a large number of sarcosomes lie close together Our Figures 22 and 34 illustrate this clearly in the vertebrate and also in insect muscle Another mechanical result of the existence of particularly large numbers of sarcosomes is that the individual muscle fibrils are forced apart by these small interposed bodies A comparison of our Figures 32 36 and 39 and in contrast Figures 33 34 41 and 42 show that in muscles which are poor in sarcosomes (such as the skeletal muscle of the mouse or of the guinea pig or of the rat or of man) the myofibrils of the muscle fiber are packed closely together whereas in the cardiac muscle of the same animal this is not the case as the sarcosomes interpose themselves be tween the myofibrils These facts emphasized by this author since 1951 are recently corroborated by pictures of Moore and Ruska (1957) of Porter and Palade (1957) and others

In sarcosome poor muscles the sparse sarcosomes are found mainly in the region of the Z zone occurring singly or in pairs (B Kisch 1955c 1956f 1957g more recently G A Edwards and co workers 1956 Porter and Palade 1957) Often where the Z bands are visible the sarcosomes appear to be attached to these bands I have therefore called these the Z form of the sarcosome or Z sarcosome for short This is illustrated for human skeletal muscle in our Figures 17 and 18 for mouse skeletal muscle in Figure 28 for guinea pig skeletal muscle in Figure 32 and for rat skeletal muscle in Figure 35 These Z sarcosomes are as a rule, much smaller than are the majority of sarcosomes found in the muscle fibers of the heart chambers although in the auricular muscle the sarcosomes are of a rather polymorphic appearance We will have more to say about this later In addition to the smaller ovoid Z sarcosomes skeletal muscle also possesses the band shaped type of sarcosomes (mitochondrial type)

In the heart as in other muscles great quantities of sarcosomes are always in the closest neighborhood of each nucleus (Kisch 1951) This seems to apply to nuclei in general for in capillary

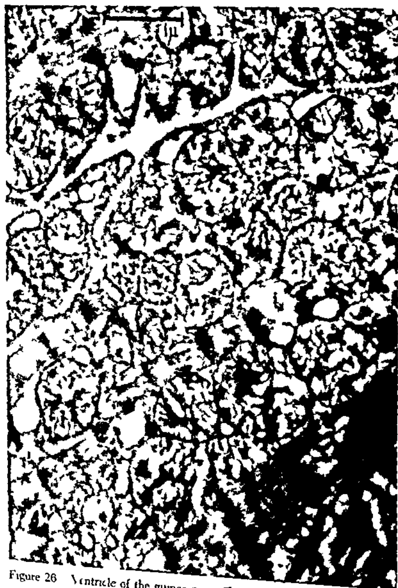


Figure 26 Ventricle of the guinea pig The mass of sarcosomes is in this picture much bigger than that of the scanty myofibrils 22 000×



Figure 27 Flight muscle of a hornet Note the large sarcosomes lying between the myofibrils, and the absence of Z bands in the presence of distinct Z M and H zones (*H zone is the light zone on either side of the M*)
SL = sarcolem T = Capillary of the tracheal system 13 000×



Figure 27a Flight muscle Honey bee Like in the heart muscle of the investigated vertebrates rows of sarcosomes fill the spaces between the myofibrils T = Capillaries of the tracheal system 14 000×

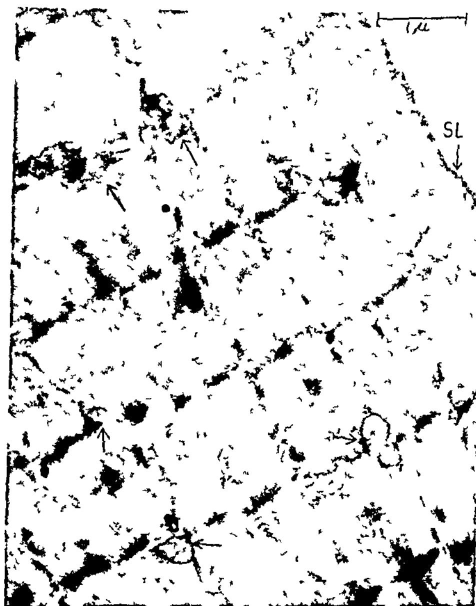


Figure 28 Leg muscle of an albino mouse The sparse sarcosomes occurring in the region of the Z zones of the myofibrils are indicated by arrows SL = Sarcolem 22 000×



Figure 29 Diaphragmatic muscle of the same animal as in Figure 28 The sarcosomes are much more numerous being partially small in the region of the Z zones and partially large lined up in rows Sarcosomes are seen in both the round and ribbon shape The ribbon shaped sarcosomes are of the mitochondrial type 15 000×

endothelium one also regularly finds plasmosomes in the region of the nuclei which would seem to point to especially high meta bolic changes within the nuclei or wherever they are found (Kisch 1951 and later) (see Figs 30 and 36)

The great quantity of sarcosomes in cardiac muscle is, pre sumably also responsible for the fact that this muscle is much harder and firmer than any other muscle in the body The great firmness of cardiac muscle was already observed by Galen who thus explained the great working ability of the heart If this peculiar consistency of the heart is as I presume the result of its enormous content of sarcosomes then Galen without ever understanding this connection was in the end quite right in his assumption

Form and inner Structure of the Sarcosomes

Thanks to electron microscopy we now recognize the fact that sarcosomes and plasmasomes including the so called mitochon dria are not merely homogenous droplets or rods of indeterminate substance of microscopic or sub microscopic size It was formerly thought that the substance of which sarcosomes are made was most probably fat glycogen or albumin The electron micro scope has shown that the sarcosomes are structures with a charac teristic external form and internal structure (Sjostrand 1953 Palade 1952b Rhodin 1954 Kisch and Philpott 1955 etc)

The recognition of the above facts enables us today to dis tinguish between sarcosomes and other microscopic and sub microscopic cell inclusions Of course the recognition of a certain inner structure is not the only method for differentiating and recognizing the sarcosomes as such in each particular case One can also draw many conclusions from the form and the location of these bodies alone Indeed it is often difficult to recognize the typical internal structure of the sarcosomes as this requires both an excellently prepared specimen and excellent photography The inner structure of the sarcosomes changes apparently with the changes of their function too

Concerning the form of the sarcosomes we must first emphasize the fact that the method of ultra thin sectioning employed in electron microscopy never allows one to see an entire sarcosome



Figure 30 Ventricle of the same mouse as shown in Figures 28 and 29 The spaces between the myofibrils are filled with sarcosomes In the region of the nucleus (N) the sarcosomes are especially numerous 13 000×



Figure 31 Auricle of the same mouse shown in Figures 28 29 and 30 Note the large number of sarcosomes between the myofibrils and also between the myofibrils and the sarcolem (SL) In the upper right hand portion of the picture there is an interstitial cell (I) containing a nucleus (N) surrounded by many plasmasomes (PS) This cell sends out its extensions to the interspace between the muscle fibers C = Capillary M = Myofibrils 7 000×

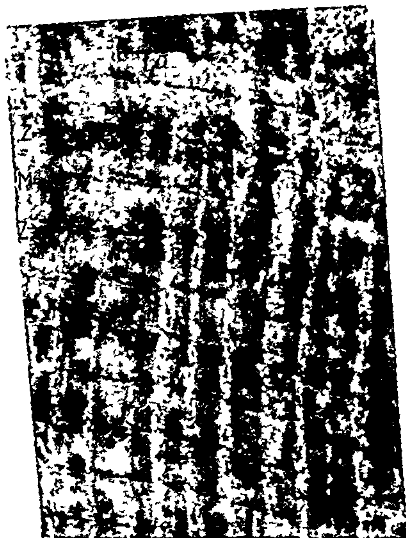


Figure 32 Leg muscle of a guinea pig It is not possible to identify a single sarcosome between the myofibrils with any degree of certainty 20 000×



Figure 33 Eye muscle from the same guinea pig shown in Figure 32 Numerous sarcosomes mostly of the mitochondrial type are found between the sarcolem (SL) and the myofibrils and between the individual myofibrils 20 000×



Figure 34 Ventricle of the same guinea pig shown in Figures 32 and 33 Masses of sarcosomes are seen lying between the myofibrils Note the double-ring form and canal form of structures between the sarcosome indicated by arrows 25 000×

Rather, what one sees in the electron microscopic image are mere slices, cut out of the sarcosome, much as slices of bread are cut from a whole loaf. Just as one could say very little about the form and size of a loaf of bread or of a sausage from seeing only a slice of it, it is very difficult to say anything about the form and size of an entire sarcosome from the picture of a single slice of the sarcosome obtained in sectioning for electron microscopy. The form and size of the slices we view are always dependent upon the plane in which the sectioning knife chanced to strike the sarcosome. The comparison of a series of many consecutive electron microscopic pictures of the same tissue, however, permits one to draw certain conclusions.

For example, if the sarcosomes of the heart chambers in the vast majority are seen to be round or oval, irrespective of whether the myofibrils between which they are found are sectioned longitudinally or transversely, then we are justified in concluding that the sarcosomes of the heart chambers, or at least the majority of them, are spherical or ellipsoid in form. If, as is occasionally the case in the auricle, the picture shows a group of very small sarcosomes lying packed close together, then we can be sure that we are dealing with small sarcosomes and not with tangential sections of larger sarcosomes, as the latter would be a geometric impossibility in the case of a cluster of such small bodies. If, as our pictures show, tightly packed sarcosomes are flattened at their adjacent edges (see Fig. 41) or, as in insect muscles, they assume bizarre forms which exactly fill out the spaces between muscle fibrils (see Fig. 22), then we are justified in concluding that the material of which sarcosomes are made up is of a plastic nature offering little resistance to a molding force.

It should also be emphasized that there is a difference in the form exhibited by the sarcosomes not only in different organs of the same animal but also in the same organ of different animals and that these differences are of a characteristic nature. Thus, as we have just mentioned, the sarcosomes in the ventricle of various mammals which we have investigated, including man, are predominantly round or ellipsoid while, as I was able to show with Philpott in 1955, the ventricle of the frog has sarcosomes



Figure 35 Leg muscle of a rat. At *only* two places within the cytoplasm between the myofibrils is it possible to see any sarcosomes (↑ × 27 000)

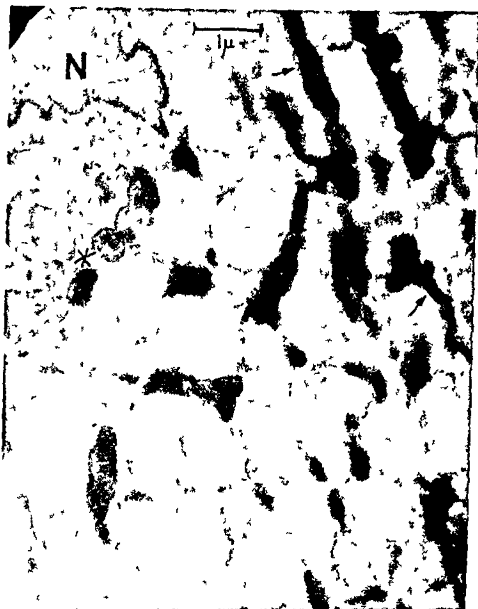


Figure 36 Diaphragm of the rat Many sarcosomes between the myofibrils Arrows indicate those of mitochondrial type Notice the group of sarcosomes at the left upper corner ( ) near to the nucleus (N) 15 000×



Figure 37 Heart muscle (ventricle) of the same rat as Fig. 36 Rows of sarcosomes between the myofibrils *Arrows indicate endoplasmic reticulum* 15 000×

which are predominantly ribbon shaped Recently Szent Gyorgyi has confirmed the same finding (1956)

This difference in the shape of sarcosomes of various tissues applies to the heart itself when we compare the auricles and ventricles of the same heart and may correspond to a different function of differently shaped sarcosomes

In *the* ventricular *musculature* for example only a small portion of the sarcosomes assumes the elongated sausage shape (mitochondrial) type *while in* the auricles many of the sarco-



Figure 38 Ventricle of the cow Masses of sarcosomes near a nucleus (N) *between myofibrils Notice their inner structure 12 000×*

somes are of this type The elongated sarcosomes can be as long as 3 μ They are generally only 0 2 to 0 3 μ in diameter The round or ellipsoid sarcosomes of cardiac muscle have a diameter of 0 5 to 1 0 μ while the sarcosomes of the insect flight muscle have a diameter of about 3 to 4 μ a fact which explains why histologists have in the past always favored these particular sarcosomes *in their investigations They are a challenge to the electron* microscopist too (B Kisch 1959) It is quite typical of the *auricles that their muscle fibers exhibit a great variation in the* form and size of the sarcosomes (Polymorphism of the Sarcosomes B Kisch, 1955 1956a b 1957g) recently corroborated for the atrium of the turtle by pictures taken by Fawcett and Selby



Figure 38a Human ventricle Sarcosomes between myofibrils arrows indicate profiles of endoplasmic reticulum 23 000×

(1958) We will later make reference to the fact that differences between the sarcosomes in the *auricle* and ventricle of the same heart support the earlier contention of this author that the biochemistry of the auricle and of the ventricle of the same heart are not necessarily identical (1956a)

The inner structure of the *sarcosome* has been variously investigated as has the structure of the so called mitochondria of the non muscular tissues such as liver kidney and glands (*Sjostrand*



Figure 39 Sarcosomes from the heart of a cow Notice their outer membrane and inner lamellar structure 30 000×

1953 Palade 1952b Rhodin 1954 Napolitano and Fawcett 1958 Kisch 1959 and others)

Palade named the lamellae which were most easily recognizable in sections of sarcosomes Cristae mitochondriales It is the hallmark of sarcosomes that they are made up of a membrane surrounding an inner content This fact had already been recognized by light microscopists investigating suitable sarcosomes (Koelicker 1886 see Kisch bibliography 1952a) In the sarcosomes of insect muscles however it is often impossible to demonstrate a distinct membrane This seems not to be an optical artifact only (Kisch 1959) In the mammal when the content of the sarcosome is removed by osmosis one can see the membrane which remains looking for all the world like an empty sac This same phenomenon is the case within a very short time after the death of these animals and this author has called these sac like empty membranes ghosts of sarcosomes (1955c) The internal lamellae of the sarcosomes which are markedly osmiophilic are



Figure 46. Sarcosomes from the heart of a guinea pig. Note the tear in the external membrane indicated by an arrow, and the concentrically arranged perpendicularly cut lamellae, of which each can be clearly recognized as having two surface boundaries. Their *width is approximately* 150 A. Their dark surface boundary is approximately 50 A thick. The two sarcosomes visible in this picture are embedded between obliquely sectioned myofibrils of the heart whose parallel protofibrils *and Z* bands are easily recognized. 58 000×

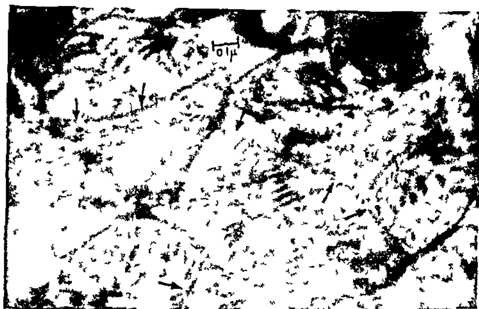


Figure 41 Sarcosomes of the guinea pig heart The arrows point to lines which might be regarded as the boundary lines of dividing sarcosomes 55 000×

closely packed together in the sarcosomes of the heart and of the flight muscle of insects On cross section they have the appearance of a double line each line having a thickness of approximately 50 to 75 Å The internal lamellae of the sarcosome surrounded as they are by a much less osmiophilic matrix, are sometimes seen to extend diagonally or transversally across the entire sarcosome At other times they are seen to be connected with the sarcosome membrane on one side only and somtimes they don t seem to touch the membrane anywhere At times, they are *arranged in parallel lines while sometimes they are arranged in* concentric circles (Kisch 1955 etc) or in radiating rays Because of this I prefer to use the term internal lamellae rather than Cristae I have often pointed out the similarity between these pictures and the trichites of Otto Lehmann (1954a 1954b and elsewhere) The content of the sarcosomes is occasionally strongly reminiscent of Lehmann s liquid crystals Occasionally however one finds no lamellae at all within the sarcosome Rather one finds rod shaped pieces of lamellae or fragmented particles within an osmiophobic homogenous matrix This may



Figure 42 Sarcosomes between myofibrils of a meat fly Notice the arrangement of the lamels in the *sarcosomes* T indicates capillaries of the tracheal system 20 000×

be the case even when neighboring sarcosomes in the same picture exhibit well developed lamellae (see Fig 39) This observation makes one wonder whether the absence of a lamellar structure is in fact only an artifact and also whether really each well preserved *sarcosome should* exhibit a regularly *ordered* lamellar internal structure It would seem much more probable that the variation in internal content is indicative of a variation in functional state of the sarcosome being viewed (Kisch 1954a 1955a c 1956 1957g lately also Powers and co workers 1958)

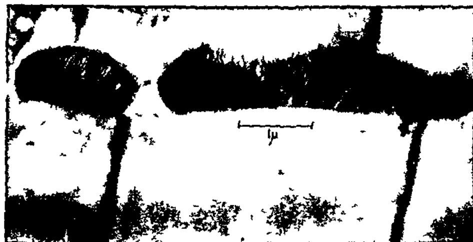


Figure 43 Sarcosomes between myofibrils of a meat fly 16 000×

or even of degenerative changes of the content of the sarcosomes which may arise under pathologic conditions

Recently the fact that neighboring plasmasomes differ, one showing the inner lamellar structure, while the next shows only an amorphous content has been confirmed also by Napolitano and Fawcett (1958) for brown adipose tissue in the new born mouse and rat These authors as well as Kisch (1959), have shown that even one individual sarcosome may in one place contain lamellae whilst in another place it contains only amorphous material All such observations seem to confirm this author's previous opinion that the observed difference in content may be due to different functional states of the sarcosomes being viewed

It should be emphasized however that necrobiosis tardy fixation after death and improper handling of the material all open the door to artifacts of swelling and disintegration which may go so far as to produce ghosts of sarcosomes I cannot go along with the concept that ultramicroscopic canals exist in the plasmasomes in which metabolic exchanges and metabolic processes occur which in turn regulate the muscle metabolism

The Function of the Sarcosomes and a Correlation Between Sarcosomes and Muscle Function

Even in the earliest electron microscopic investigations of sections of cardiac muscle (Kisch 1951 1952) the most striking



Figure 44 The internal structure of the sarcosomes of a wasp caught in the summer time The sarcosomes have a striking corrugated and wavy internal lamellar pattern The letter T denotes a branch of a capillary of the air transporting tracheal system (From Kisch and Philpott 1955) 34 000×

feature was the enormous quantity of sarcosomes within the muscle fibers of the heart After examining countless pictures I would today somewhat allegorically state the case as follows whereas in the electron microscopic picture of cardiac muscle one often has to search amongst the sarcosomes for the individual muscle fibrils in the picture of skeletal muscle one must search



Figure 45. Sarcosomes from the human ventricle. Note the double limits of the inner lamels. (From Kisch, Cavusoglu and Marangoni, 1959.) 70,000×

among the miofibrils to find sarcosomes. A simple comparison of Figures 28 through 38a showing both cardiac and skeletal muscle of the same animal will make this point quite clear.

All biochemical studies of cardiac muscle should also in future take cognizance of the fact that at least in certain places cardiac muscle contains a mass of sarcosomes which is equal to or outweighs the mass of myofibrils.

When working chemically on extracts of heart muscle one should be aware of the fact that *the material which is extracted is not only muscle fiber*. Rather, in places the extract is made up

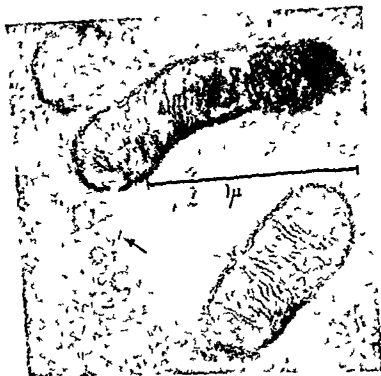


Figure 46 Plasmasomes from a thrombocyte of the mouse The arrow points to cross sectioned channels in the cytoplasm Notice the membrane of the plasmasomes and the double lining of the inner lamels 58 000×

of 50 % or more sarcosomes or their extracts only the remainder being extracted of myofibrils *Extracts of heart muscle therefore do not permit of comparison with extracts of skeletal muscle* for the purpose of deriving any conclusions about existing differences in the chemical contents of the myofibrils of these two types of muscle (Kisch 1956f 1957g)

In line with the above stated condition of the human heart muscle we should take into consideration one other fact In nature a similar occurrence of a huge mass of sarcosomes in which myofibrils are embedded is found only in the flight muscle of insects Linking these two facts together I came to the conclusion in 1951 that sarcosomes must be essential to the intensive and ceaseless activity of these two otherwise seemingly unrelated muscles Originally one had suspected that the sarco

somes were either depots for energy rich material used by the strenuously and steadily exercising muscles, or else fatty degeneration products of the muscle fiber itself (see Kisch 1952a) In recent years however, various researchers have demonstrated for the mitochondria of other organs (liver, kidney), that their plasmasomes are in reality enzyme depots (literature, G H Bourne 1950) *This seemed to me to be the answer to one of the central problems of cardiology* I assumed (1951) that the sarcosomes of the heart contained enzymes of the most vital importance to the heart s metabolism The vast numbers of sarcosomes present in cardiac muscle compared to skeletal muscle together with their advantageous position within the muscle fibers would seem to make them ideally suited for effecting an immediate reversal of the chemical changes which occur in connection with each contraction of the cardiac muscle This is more especially so as the usefulness of an enzyme within a cell undoubtedly depends upon its quantity and its localization within that cell As we can see both the location and the quantity of sarcosomes within cardiac muscle are optimal The mass of the sarcosomes exceeds the mass of the myofibrils at many places within the ventricle and all the sarcosomes are found to lie within the muscle fiber It is because of this that under normal conditions one never finds bigger amounts of metabolites as chemical exchange products accumulating within the heart muscle Therefore in contrast to normal skeletal muscle which possesses relatively few sarcosomes the normal heart can carry on strenuous work for a lifetime without requiring any lengthy pause to recover (Kisch since 1950)

Thanks to the outstanding work of biochemists the importance of this opinion has subsequently been thoroughly substantiated for the sarcosomes of both insect muscles (Watanabe and Williams, 1951 1953) and cardiac muscle (Plaut and co workers 1952 Slater and Cleland 1952 Cleland and Slater 1953a b Siekevitz 1959) *Sarcosomes are carriers of metabolic enzymes and by means of their enormous quantity and favorable location within the heart they permit that organ to function without any lengthy rest period through an entire lifetime* The reader is referred to the above mentioned references for biochemical details

It is as yet too early to state definitely the mechanism by which the enzymes are released from the sarcosomes It might be that part of the enzymes are located at the surface of the lamellae within the sarcosome and that they are released in response to biochemical and physicochemical changes in their environment Or they might be located at the sarcosome membrane In each case they may leave the sarcosomes by osmosis under favorable circumstances It is also conceivable that both of these mechanisms may prevail to some extent As previously mentioned the double interphase boundary of the lamellae which can be easily recognized in the pictures has a thickness of approximately 50 to 70 Å This could correspond to the thickness of macromolecules of osmiophilic enzyme proteins or enzyme lipoproteins which are located in these particular places Whether the appearance of the internal structure of the sarcosomes changes with their enzymatic activity and whether perhaps these organelles might play a recognizable part in clinically proved insufficiency states of cardiac muscle is the present topic of our investigations (Kisch 1954 1959) All of the above are new approaches towards the solution of important clinical problems in which experiment and electron microscopy together would seem to hold the ultimate answer Upon the solution of these problems will depend the further development of many phases of cardiology Of one thing we can be certain even at this point within a very short time after death the sarcosomes lose their content and only the empty membranes remain giving rise to the so-called "ghosts of sarcosomes (Kisch 1953)

A first attempt at solving this type of problem has led to the following histological results which are of basic importance

Cardiac muscle and skeletal muscle are for all practical purposes two ends of a spectrum Cardiac muscle works day and night without longer rest periods against the resistance of the blood pressure within the aorta and the pulmonary artery In some small animals (such as the mouse the rat the hamster the sparrow the finch and the sea gull) the heart beats at a rate of 300 to 1000 contractions per minute (Kisch 1953a) In contrast to cardiac muscle skeletal muscle works under a requirement of substantial periods of rest When such rest periods are not ex

tensive enough skeletal muscle tires very easily. As has been pointed out, in line with this difference in function, cardiac muscle contains a very considerable quantity of sarcosomes while skeletal muscle contains very few. Among the striated muscles of the vertebrates, there are many which classified according to function would fall in between cardiac and skeletal muscle as described above. For example, the eye muscles function continuously while one is awake and the diaphragm functions continuously day and night. Naturally, the resistance against which the diaphragm must work is minimal when compared to the resistance offered the cardiac muscle. Electron microscopic studies have now revealed (Kisch 1956e, f, 1957g) that just as the function of the eye muscles and diaphragm falls in between the two extremes of cardiac and skeletal muscle function, so also their content of sarcosomes falls in between the extremes. Their muscle fibers, especially those of the diaphragm, contain many more sarcosomes than those of skeletal muscle and yet their sarcosome content falls far short of that of cardiac muscle of the same animal. (See Figs. 28 through 38a.) The cardiac muscle of varying animals also exhibit typical variations in their sarcosome content. In the hearts of cold blooded animals (fish, frog) I found far fewer sarcosomes than in the hearts of mammals (Kisch 1954a), and in the ventricle of cattle (pulse rate 30–50) far fewer than in that of man or small mammals.

The statement about the astonishing abundance of sarcosomes in the diaphragm (Kisch 1956e, f) has recently been confirmed by a picture of the muscle of the diaphragm of the rat published by M. L. Watson (1958).

All of these observations point out one basic fact: *the ability of a striated muscle to do strenuous untiring work is in mathematical terms a function of the number of sarcosomes which it contains* (Kisch 1951, 1954a, 1956b, f, 1957g) and of the number of functioning capillaries per mm^2 of the muscle. The vital importance of the amount of sarcosomes in the muscle fiber is apparently still not well understood even by most of the scholars working on sarcosomes.

Finally, it should be mentioned that perhaps the varying forms of the sarcosomes which we observe may also signify varying



Figure 47 Section through a red blood cell of man No plasmasomes and no endoplasmic reticulum noticeable 36 000×

function After all we are used to the idea that in nature generally difference in form signifies difference in function It has already been mentioned that part of the sarcosomes of the auricle exhibit a different form and a different distribution than do the sarcosomes of the ventricle of the same heart The typical sarcosomes of skeletal muscle the Z-sarcosomes are decidedly

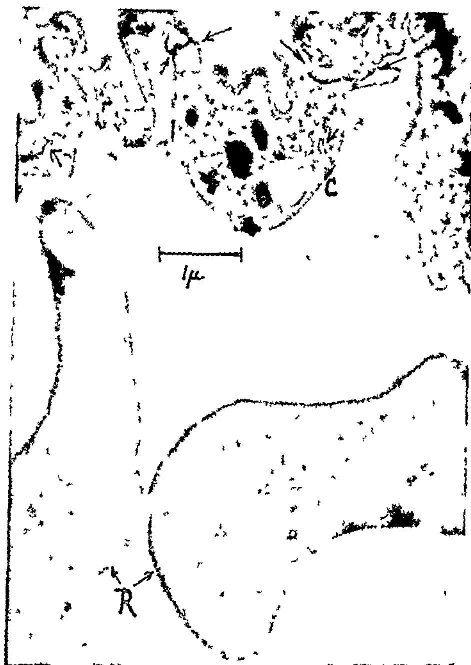


Figure 48. 2 Red Blood cells (R) in capillary of the human heart. No plasmasomes and no endoplasmic reticulum. In the capillary wall (C) a big vacuole, different plasmasomes and many small droplets. Arrows point to parts of endoplasmic reticulum and 2 slits in the wall. (See p. 119 ff.) 18 000 x



Figure 49 Capillary from the heart of an albino mouse Within the cell wall the endothelial nucleus (EN) the mural slit (WS) and the plasmasomes indicated by arrows are visible The capillary closely surrounds a white blood corpuscle whose nucleus and plasmasomes are easily recognized 13 000×

smaller than are the sarcosomes of the ventricle and they are mostly located singly or in pairs at the Z zone

We are still in need of a detailed study to allow inclusion within this picture of the most varied of animals and of the nonstriated muscles too Our own as yet unpublished comparative studies point towards the fact that for example the internal structure of the sarcosomes of the flight muscles of insects may be dependent upon the season This could be a factor in the decreased flying ability of certain insects in the fall of the year (e g flies)

Electron microscopic studies of the sarcosomes have led since 1951 to investigations which *with surety have proven that these structures are a vital part of the cardiac muscle* It is however *not this writer's opinion that the enzymes of the cells* are located and built exclusively in *plasmasomes* (mitochondria)

In connection with the above, we should recall here that large numbers of plasmasomes are also found in the region of the nerve end plates of striated muscle as W Kuhne has already shown decades ago In line with current thinking about the method of transmission of nerve impulses I thought it likely (1952a) that the piling up of plasmasomes in this location was somehow connected with the production of the acetylcholinesterase which is required there This close connection between plasmasome content and function of a cell can also be demonstrated in many other fields of physiology which can be mentioned here only in passing

Of all the cells in the body the mature red blood corpuscles of mammals probably have the lowest oxidative chemical exchange and the lowest oxygen consumption This is commonly connected with the fact that mature red blood corpuscles do not have a nucleus This could well be a factor in the unique ability of the red blood corpuscles to transport oxygen As far as we know however the red blood corpuscles of mammals also have no plasmasomes (mitochondria) while in contrast the white blood corpuscles of the same animals have nuclei and a vast quantity of plasmasomes The lack of plasmasomes in mature red blood corpuscles of mammals must be another especially important factor in the low chemical exchange of these cells The immature blood cells of the mammal (the reticulocytes) have a distinct oxygen consumption (Ramsey and Warren 1932) and they also possess plasmasomes of both a typical and an atypical variety (Chalfin 1956 Brunner and co workers 1956 Braunsteiner and co workers 1956 this author s unpublished observations) One other thought comes to mind O Warburg demonstrated the abnormal oxygen consumption of tumor cells A study of the plasmasomes of tumor cells could reveal as yet unknown morphological characteristics of these cells

All the above raises the question of the relation between the nucleus and the plasmasomes of a cell Since 1951 I have stressed the fact that numerous plasmasomes (sarcosomes) are always found in proximity to the nucleus In mammalian red blood corpuscles both the nucleus and the plasmasomes are absent Can not one of these two structures exist without the other? Can the



Figure 50 A red blood corpuscle and a white blood corpuscle in a capillary of mouse lung Within the white blood corpuscle are seen two sections of the nucleus (N) and numerous plasmasomes of which several are indicated by arrows There are no plasmasomes seen in the red blood corpuscle 11 000×

plasmasomes exist only if a nucleus is present? This is doubtful according to the findings in blood platelets Can the nucleus exist only if the plasmasomes are present? This is perhaps more probable Perhaps though both the nucleus and the plasmasomes disappear simultaneously in the course of the development of the red blood corpuscle which also has no endoplasmic reticulum It will require much work before one will be entitled to a definite opinion concerning the answer to these questions In

In connection with the above we should recall here that large numbers of plasmasomes are also found in the region of the nerve end plates of striated muscle as W Kuhne has already shown decades ago In line with current thinking about the method of transmission of nerve impulses I thought it likely (1952a) that the piling up of plasmasomes in this location was somehow connected with the production of the acetylcholinesterase which is required there This close connection between plasmasome content and function of a cell can also be demonstrated in many other fields of physiology which can be mentioned here only in passing

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myofibrils or near the nuclei where ordinarily masses of normal sarcosomes can be found in the muscle fiber

These findings not only prove the suspected role of the sarcosomes in the failing heart but give cardiology an entirely new concept concerning the mechanism of heart failure

This author regards disintegrating sarcosomes also as a source of lipofuscine and some other lipids found by light microscopists in the heart of cardiacs and old people (See Chapter XIX)

this connection, it would be very useful to have histological studies made upon the nucleus containing erythrocytes of amphibians, fish, and birds Within the nucleus containing red blood corpuscles of the frog and fish I was actually able to demonstrate the existence of plasmasome like inclusions as well (unpublished investigations and 1957g) This observation has recently (1959) been confirmed for the eel (Anguillula Japonica) by N Tsumura

It is a well known fact that the mature red blood corpuscles are the only cells of the body of humans and of other mammals which contain no nuclei *It is a fact that they are also the only cells of the body which possess no plasmasomes (mitochondria) and no endoplasmic reticulum*

Details of the internal structure of sarcosomes are demonstrated in Figures 39 through 46, while Figure 26 demonstrates impressively regional massing of sarcosomes, between the myofibrils of the guinea pig heart (left ventricle, near the origin of the aorta)

It should also be mentioned that within the interior of sarcosomes one can frequently see dark homogenous spots which I have upon occasion designated as nucleoli of sarcosomes (Kisch 1953c Kisch and Philpott 1953)

We should also point out the fact that as in our Figure 39 one frequently finds adjacent to sarcosomes with well developed internal lamellae sarcosomes with either a completely homogenous content or a content which looks to be crumbly Perhaps this observed difference could be related to a different functional state occurring in adjacent sarcosomes as mentioned above or to abnormal conditions

The final proof of this author's concept about the sarcosomes and their vital importance for the heart was recently found in fresh specimens of the human heart of patients with chronic cardiac failure taken immediately after death (Kisch *et al* 1959 1960a and b) A great part of the heart's sarcosomes in each one of ten investigated patients was swollen or its content degenerated into a strongly osmiophylic crumbled mass Finally these sarcosomes disintegrated entirely and only crumbs of dark material indicate in these pathologic hearts the place between the



Figure 51 Human auricle The arrows indicate loop shaped structures of undetermined significance lying between myofibrils whose parallel protofibrils are easily recognizable and sarcosomes which are but poorly visualized 35 000×

X

MEMBRANES AND OTHER STRUCTURES WITHIN THE CARDIAC MUSCLE FIBER

In addition to the sarcosomes one can recognize other formed elements within the protoplasmic matrix occupying the space between the myofibrils of the cardiac muscle and that between the myofibrils and the sarcolem. We have already made mention of some ring shaped structures (see Fig. 25) (Kisch 1956e). Similar structures have been found within the human ventricle (Kisch 1960) and atrium (Fig. 51). We should also make mention of the membrane which appears in pictures of sectioned tissue as bounded by two lines and which is occasionally found with such frequency that one cannot assume this structure to merely represent sectioned pieces of sarcolem (1954a). In our Figure 52 for example one can see within a region approximately 4 μ in diameter no less than five such wavy membranes all running nearly parallel to one another. Similar lamellae can also be found within the muscle fibers of the human auricle (see Fig. 51). I have in addition occasionally found branching structures of the finest order of magnitude occurring between the myofibrils of cardiac muscle. These structures are demonstrated in our Figure 53 which shows the cardiac muscle of a bat (Kisch, 1954a).

An amazing structure is illustrated in Figure 54. This figure is taken from the auricle of a guinea pig, demonstrating a row of concentrically arranged lamellae not surrounded by any common membrane lying between the sarcosomes of the auricle. This particular structure was found in the space between the sarcolem and the myofibrils (Kisch 1956a, c). Small grains of the finest magnitude are seen attached to the individual lamellae. The whole structure suggests in its appearance the so called endo



Figure 53 Section through the ventricle of a bat Myofibrils with sarco somes interspersed between them The arrow points to a branching canal which is running towards a myofibril It is not certain whether this canal belongs to the endoplasmic reticulum (From B Kisch 1954a) 33 000×



Figure 52 Section through the cardiac muscle of a pipe fish The numerous membranes between the myofibrils indicated by arrows are noteworthy (From B Kisch 1954a) 27 000×



Figure 53 Section through the ventricle of a bat Myofibrils with sarcosomes interspersed between them The arrow points to a branching canal which is running towards a myofibril It is not certain whether this canal belongs to the endoplasmic reticulum (From B Kisch 1954a) 33 000×



Figure 54. Concentric lamellae found between the sarcosomes of a guinea pig auricle. Tiny bubbles are seen adherent to the lamellae. (From B. Kisch 1956a.) 47,000×

plasmic reticulum which is found in other tissues (see Palade 1956).

Again this entire field requires extensive further investigations. The endoplasmic reticulum found in the muscle fibers as well as in other organs of the body is not at all clarified in regard to function as yet. As a matter of fact it has not yet been proven that the endoplasmic reticulum is in reality a true reticulum, that is, a real web structure. It might perhaps be more



Figure 55 Atrium of the cow's heart. Sarcosomes between myofibrils. C = Cytoplasmic material. ER = Profiles of endoplasmic reticulum. 12 000×

correct to designate the structure as an ultramicroscopic system of canals of changing outline and content. The question has recently been raised as to whether this so called "reticulum" is perhaps identical with the ergastoplasm of earlier histology. Also the possible relation of the reticulum to the so called Golgi apparatus has been indecisively discussed at various times (Dalton and Felix 1956, Palade 1956).

I would like to confine myself here to the photographic demonstration of membranes and rods or lamellar structures as

they have been observed up *to now* within cardiac muscle, while avoiding theoretical speculation as to the significance of these structures We need to stress only one point: these well delineated structures are definitely not artifacts but rather they are naturally occurring ultramicroscopic structures in the heart and they occur in human cardiac muscle as well (see Fig 51) Probably they are not identical to those diffuse accumulations of liquid which were designated as lacunes cisterns, etc Whether in life all of these spaces are also well demarcated by membranes as they are in the electron microscopic image and especially in diagrams should not be decided as yet In my opinion the lacunes and the entire so called endoplasmic reticulum are a liquid part of the cytoplasm having fluctuating boundaries and continuously changing contents differentiated from other parts of the cytoplasm probably by physical chemical and physico chemical properties The time has not yet arrived to attribute a *physiological function to such structures* as so little is known about them It should only be emphasized that the mature erythrocytes of mammals are probably the *only cells* of the body which contain no nucleus no plasmasomes (mitochondria), and no endoplasmic reticulum as I have been able to demonstrate (See page 112 for the endoplasmic system of channels in Purkinje s fibers)

A possible relationship between but not identity of endoplasmic *reticulum and finest droplets present in endothelial* cells of proto capillaries in musclefibers of the heart in nerve axons and in other cells should be considered They probably play an important *role* in the exchange of material between the cells and the intercellular space (see our Fig 78 from the human ventricle) Warren Lewis called (1931) the drinking of droplets by cells pinocytosis H Stanley Bennet has discussed droplets as a type of Membrane flow (1956) I call this droplet transfer dropletdialysis in contradistinction to molecular transfer

Whatever one s opinion may be concerning these and other ultramicroscopic membranes lacunes and droplets it seems (1952b c) that electrophysiologists must of necessity take serious cognizance of all these morphological entities when formulating their

own explanations attempting to make use of Bernstein's membrane theory. It is poor practice to dispose of factual organic entities by means of a few words. In reality, where within the heart is this membrane of which the theoreticians of electrophysiology tell us? Is it perhaps the surface of a group of musclefibers, or the sarcolem, or even perhaps the surface of the myofibril which, although it is not an organic membrane, represents an interphase (*Phasengrenzschicht*)? What role, if any, do the above mentioned various intrafibrous membranes play in the electrophysiology of the heart, and what roles are played by the membranes which surround the sarcosomes and, lastly, by the *Z* bands which traverse the muscle fibers?

Only recently does this consideration, which the author has emphasized since 1950, seem also to be shared by others (Moore and Ruska 1957).

XI

INTERCALATED DISCS

Cardiac muscle is transected perpendicularly to its long axis by fine striations, which are partially arranged in step wise fashion and which *partially transect the muscle in a straight line*. These striations are known as intercalated discs in the English literature. In the German literature they are referred to as *Glanzstreifen, Kittlinien* or *Schaltstücke*. In the French literature they are referred to as *bandes scalariformes*. The width of these discs and the frequency of their occurrence varies with the species of animals studied, with the age of the animal and within the same heart with the area sectioned. Apparently, they are found particularly frequently in older individuals in the papillary musculature and in the left ventricle; by contrast they are found less frequently in the auricles.

Since the time (1951b) of the first attempt to demonstrate intercalated discs electron microscopically, various studies into the ultrastructure of these entities have appeared. In 1953 V. L. van Breemen investigated the intercalated discs of the dog heart and concluded that the electron microscopic pictures of his studies substantiated the theory repeatedly stressed in the light microscopic literature that the intercalated discs represented the boundary between two neighboring cardiac muscle cells. He considers it significant to this concept that those pictures which are designated as representing a section through an intercalated disc all contain a line which is in direct continuity with the sarcolem which line represents the boundary between individual cardiac muscle fibers as well. Van Breemen also emphasizes the fact that in his experience the myofibrils of the muscle fiber never traverse the intercalated discs. Rather the myofibrils end at the discs and

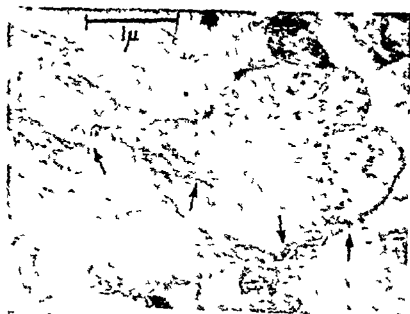


Figure 56 Auricle of the mouse Arrow points to what is probably part of an intercalated disc 25 000×

therefore the intercalated discs do not represent an intracellular structure He regards the intercalated discs as a corrugated separator between the muscle cells made up of two layers of membrane which in turn represent the boundaries of two adjacent heart muscle fibers A paper by Poche and Lindner (1955) agrees almost completely with the findings and the opinions of van Breemen as does a study by Sjostrand and Anderson (1954) Kisch (1956a) Lindner (1957) Moore and Ruska (1957) Muir (1957) and Lanzavecchia (1957)

The investigated animals included mammals frogs and chicken embryos

The present work contains two illustrations (Figs 56 and 57) in which similar membranes running in zigzag fashion are seen in the auricle and ventricle of the guinea pig I have found membranes of very similar form in freshly fixated human auricular tissue and in the heart of the pipefish (1954a) Despite this it would seem necessary to continue these investigations further before one can wholeheartedly endorse the interpretation given to

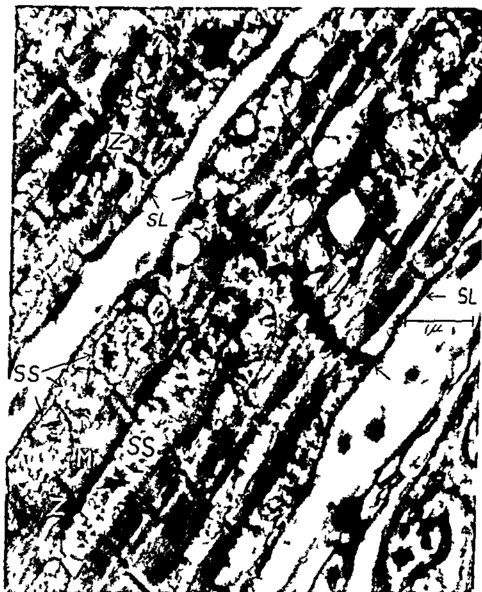


Figure 57 Left ventricle of the guinea pig heart A zigzag line cuts directly across a muscle fiber and is connected with the sarcolem SL The myofibrils with their characteristic striations and the sarcosomes SS are easily visible *The myofibrils do not seem to be interrupted by the perpendicular line* 17 000×



Figure 58 Intercalated disc (↑) in the cows atrium 13 000×

all these zigzag lines by the above mentioned authors It should be noted however that in all of my corresponding pictures one can also see a clear connection between the zigzag lines and the sarcolem This would seem to strengthen the above author s theory that the intercalated discs represent cell boundaries

In contrast however I would like particularly to call attention to Figure 57 Should the strongly osmiophilic zigzag line pointed out by arrows in this figure actually represent an intercalated disc then there can be no doubt that the individual myofibrils are not entirely interrupted by this line which connects with the sarcolem Rather the myofibrils continue across the line and beyond

In recent investigations the same type of intercalated discs have been found in Purkinje s fibers of the ventricle (not yet published own observations)

Another remark seems to be in place here Recently the ultramicroscopic structure of the intercalated discs and their connection with the sarcolem has been used as proof that the heart muscle is not a syncitium (Poche and Lindner 1955 Lindner 1957 Muir 1957)

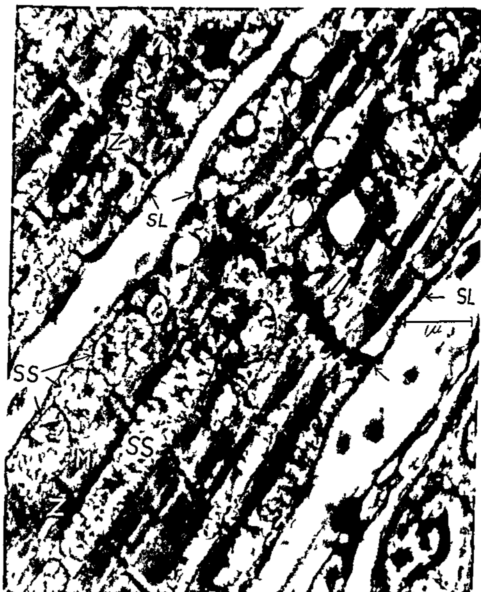


Figure 57 Left ventricle of the guinea pig heart A zigzag line cuts directly across a muscle fiber and is connected with the sarcolem SL The myofibrils with their characteristic striations and the sarcosomes SS are easily visible The myofibrils do not seem to be interrupted by the perpendicular line 17 000×

XII

THE NUCLEI

Light microscopy has known for a long time that the nuclei of the cardiac muscle fiber are predominantly located in a central position. This is in contrast to skeletal muscle where the nuclei are predominantly located towards the periphery, that is, in close proximity to the sarcolem. The number of nuclei found in the embryonic heart is markedly in excess of the number found in the heart of the adult animal. This observation previously made in the mouse (Kisch 1951) can now be confirmed in the case of the guinea pig and the human (author's unpublished studies).

In the ventricle the nuclei of the muscle cells assume, as a rule, an elongated ellipsoid form with the long axis running parallel to the myofibrils. Whenever the nuclei of ventricular musculature appear round in a photograph the most probable explanation is that the photograph represents a cross section through a nucleus rather than that the nucleus is in actuality spherical. Very frequently the nuclei appear to have a bizarre form (Figs 60, 61 and 62). Sometimes they also seem to be deeply indented. If a section cuts across such an indentation or several of them the resulting picture may seem to show one large nucleus and in addition one or more smaller nuclei (Fig 62), whereas in reality we are dealing here only with one or more additional pieces of the same nucleus (Figs 59 and 62). As repeatedly pointed out since 1951 one always finds a collection of sarcosomes in close proximity to the *nucleus* of cardiac muscle and a collection of plasmasomes in close proximity to each nucleus in the endothelium of blood vessels.

Since it has been shown that the function of the sarcosome is to provide for the tissues an available source of necessary enzymes

This author has emphasized repeatedly since 1950 that the ultramicroscopic inner structure of the muscle fiber and the presence of its sarcolem excludes the concept of its being a syncitium (1951, p 25 1952c 1957g and so on)

Concerning intercalated discs in the human ventricle see Chapter XIX (Fig 95)

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Concerning intercalated discs in the human ventricle see Chapter XIX (Fig 95)



Figure 61 Nucleus from a Purkinje fiber of the cow's heart Note the spongy structure of the three nucleoli 14 000×

for chemical exchange the above observation is an important indicator that *the nucleus even when it is not dividing has a constant sizable rate of metabolism* (Kisch 1951)

Every nucleus exhibits a thickened outer bordering layer the nuclear membrane and one or more nucleoli The nuclear membrane often has chromatin fragments attached to its inner surface Perhaps these chromatin fragments represent post mortem or necrobiotic phenomena as they rarely occur in satisfactorily fixed nuclei with uniformly distributed contents Because of the ultra thin sections made possible by today's technology we now occasionally see pictures of nuclei depending on the plane of sectioning which contain no nucleoli at all (Figs 59 and 62) With

7762.

Figure 59 Bizarre form of the nucleus in a human ventricle 13 000×



Figure 60 Nucleus from the auricle of a guinea pig. Note the spongy appearance of both nucleoli 13 000×

fibrils At other times masses of sarcosomes crowd in between the nucleus and the myofibrils Occasionally one sees the nucleus lying in a wide environment of cytoplasm (endoplasm) surrounded by many sarcosomes We have not been able as yet to demonstrate any typical differences between the nuclei of the auricles and those of the ventricles However it would seem that the elongated ellipsoid nuclear shape is more prevalent in the muscle fibers of the ventricle whereas the auricles not infrequently possess the more bizarrely shaped type of muscle nuclei (Fig 59)



Figure 62 Deeply indented nucleus from the human heart surrounded by sarcosomes (SS)

thicker sectioning one rarely sees nuclei in the heart muscle which do not contain at least one and usually several nuclear inclusion bodies These bodies are not merely solid spots dark through their ability to absorb osmium stain but rather *the nucleoli exhibit very often a coarse sponge like inner structure of their own* (Figs 60 and 61) The rest of the nuclear content present in appearance of more or less homogeneous material or granulation depending on the quality of fixation and upon other processing of the tissue Sometimes the nucleus is packed tightly between muscle



Figure 63 Interstitial cell in guinea pig heart Note the long thin extension of the cytoplasm running along the sarcolem of a neighboring muscle fiber (From B Kisch 1956a) 8 000×

Figure 64 Human auricle A cell found between muscle fibers The protoplasm is filled with tiny strongly osmophilic droplets giving the entire cell an appearance of having been sprayed with confetti 17 000×

XIII

INTERSTITIAL CELLS

In the space between the individual muscle fibers of the heart we find vessels, nerves and certain types of cells which shall be discussed now.

The first type of cells found here are the pericytes or Rouget's cells which in part are found directly adjacent to the capillaries. These cells will be the subject of a later chapter (see p. 128 ff.). In addition one finds cells in the space between the muscle fibers of the heart which most probably belong in the category of connective tissue cells. These interstitial cells stretch by means of wing shaped or lamella shaped extensions between the sarcolem of adjacent muscle fibers. They have large nuclei and by comparison a scanty compact quantity of protoplasm with far reaching lamella shaped extensions. Plasmasomes are found within their protoplasm in proximity to the nucleus. In the electron microscopic picture of ultra thin sections the fine lamella shaped extensions of these cells have an appearance identical to sections through membranes which extend between two individual muscle fibers. Only in areas where, as in our Figure 63, the connection between these membranes and the protoplasm of the interstitial cell can be shown does the true identity of these interfibrous membranes become clear. Such membranes can also surround entire groups of cardiac muscle fibers in which case they may become what in an earlier chapter (Figs. 5 and 6) was referred to as a sarcolem of the second order. These facts can be best seen in the ultramicroscopy of teased tissue preparations.

Low (1953) has demonstrated for epithelial cells of the lung alveolus that the extensions of these cells can run in an unimaginably thin layer along the surface of alveoli covering the capil-



Figure 63 Interstitial cell in guinea pig heart Note the long, thin extension of the cytoplasm running along the sarcolem of a neighboring muscle fiber (From B Kisch 1956a) 8 000×

Figure 64 Human auricle A cell found between muscle fibers The protoplasm is filled with tiny strongly osmophilic droplets giving the entire cell an appearance of having been sprayed with confetti 17 000×

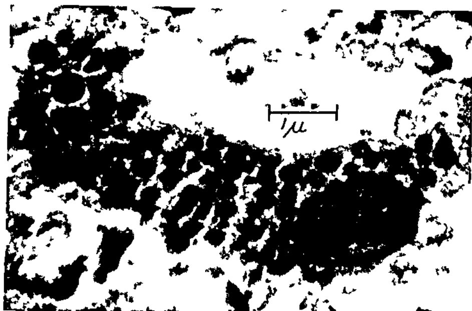


Figure 65 A cell similar to the one shown in Figure 64 This cell was obtained from sections through the auricle of another human patient suffering from rheumatic valvular heart disease 15 000×

laries Similarly, fine extensions of the interstitial cells stretch, as I have been able to show between the individual cardiac muscle fibers as well (1956a 1957g see also Fig 63) This particular arrangement undoubtedly has physiological significance in the cardiac muscle although at the present time this significance is still unknown to us

These observations in the lung and in cardiac muscle make it requisite that histologists and physiologists become aware of the fact that the individual cells in the tissue of a living organism are not simply compact masses of closely grouped entities (nucleus with surrounding protoplasm) Rather the protoplasm of these units is in many cases connected with the space in between the neighboring cells by means of pseudopod extensions Light microscopy dependent as it is upon the use of stains is not able to demonstrate for us properly these findings which seem significant to an understanding of the positioning of the individual cell within a tissue I was able to show similar conditions to exist in endothelial cells of the smallest airducts of the lungs (1958b)

The above observations also help us to understand the structure

of the simplest capillaries of which we will speak in more detail in a later chapter Lamella shaped extensions of the endothelial cells which make up the walls of the simplest capillaries expand and surround the capillary blood stream in a tube like manner In those places where the extensions finally meet and overlap in the periphery there arise the mural slits of the capillary tube which I have repeatedly described (1955a 1956b) and will describe further in the next chapter

I would still like to mention one *special* type of cell which I have occasionally found between the cardiac muscle fibers In preparations of the dilated auricle of two human hearts containing old rheumatic valvular defects I found a unique type of cell whose entire protoplasm was studded with osmiophilic drops of *finest dimension* (see Figs 64 and 65) These osmophilic drops exhibit no internal structure which might allow them to be labeled as plasmasomes They are rather homogeneous ultra microscopic droplets made up of lipid or some other osmiophilic matter I cannot at the present time say whether these cells are a *pathological* manifestation *or not* Neighboring cells within the tissue of *these* two hearts did not exhibit the unique traits enumerated above I have occasionally seen large quantities *of similar osmiophilic* droplets in the endothelial cells of the auricle of the bat In *these bats* as in the humans the appearance of the *osmiophilic* droplets was restricted solely to one type of cell while *neighboring cells* in the deeper cell layers contained no droplets

Interstitial *cells of another* kind are found near capillaries in the human ventricle (Kisch 1960b) They contain many ghosts of mitochondria and an extremely widened endoplasmic reticulum *Their appearance* reminds one of plasma cells See such an E R cell Chapter XIX (Fig 97 and 98)

XIV

THE ENDOCARDIAL CELLS

The endothelial cells of the inner surface of the heart have not yet been extensively investigated with the electron microscope. Some isolated observations on the auricle of the guinea pig have already been published (1956a). More recent observations are in agreement with these earlier findings. As seen in Figure 66 the endocardial cells have large nuclei and their basilar membrane bounds on a layer of very fine granular material which is in direct contact with the sarcolem of the outermost muscle fibers. I called it the sub-endothelial, or sub-endocardial, stratum.

One is struck with the fact that certain of these endothelial cells, especially in the embryonic heart, are extremely rich in plasmasomes. Since one can generally demonstrate that tissues with active chemical exchange are also rich in plasmasomes, the above observation would seem to indicate that the endothelial cells of the heart possess a very active metabolism and therefore most probably have an important, although as yet undetermined function. We are at present engaged in a special study of this component of cardiac muscle.

The many unmyelinated nerve axons which can be found in the subendothelial stratum between endothelium of the heart and sarcolem are probably the afferent branches of the Bezold-Jarisch reflexes (Kisch 1956b, 1957g). Their existence has recently been confirmed by electron microscope pictures of the atrium of the turtle (Fawcett and Selby 1958).



Figure 66 Endothelial cells from the auricle of a guinea pig These cells containing large nuclei have their basal membranes and cell boundaries indicated by arrows Beneath them there is a finely granulated layer the subendothelial stratum with inclusions the latter indicated by an X These inclusions represent nerve axons The granulated layer bounds directly on the sarcolem (SL) of the outermost muscle fiber (From B Kisch 1956) 10 000×



Figure 67 Endocardial cells from the ventricle of the rabbit Big nuclei and scanty plasmasomes 8 000×



Figure 68 Endocardial cells from the heart of a cow (ventricle) Big nuclei with plasmasomes in their neighborhood and profiles of a system of smallest channels 9 000×

XV

THE CONDUCTIVE SYSTEM (PURKINJE FIBERS)

The history of the discovery of the stimulus conducting system in the heart can be divided into two phases In the early Nineteenth century (1846) the Breslau physiologist, J E Purkinje discovered within the ventricles the subendocardial system of specific fibers, which still today bear his name

Purkinje of course did not yet realize the physiological significance of this structure At the end of the Nineteenth Century (1893), W His Jr discovered the muscle bundle which connects the auricles with the ventricles and recognized the physiological function of this structure generally referred to as the Bundle of His L Ashoff and his collaborators, particularly Tawara (1906) then studied the ramifications of the conductive system (Tawara's Bundle Branches) whose finest subdivisions are Purkinje's fibers

Textbooks of histology usually mention the bundle of His and its branches only as a kind of succulent muscle fiber rich in protoplasm

It seemed enticing to study this kind of tissue with the electron microscope

As material, the author used Purkinje's fibers and the chordae tendineae spuriae of cattle and rabbits especially the ribbon traversing the right ventricle (moderator band)

These investigations of the conductive system which are by no means as yet completed have thus far yielded the following results

Whenever one of the smallest peripheral trabeculae also known as Purkinje's fibers or one of the bigger false chordae tendinae is fixated in 1% Osmium Ringer's solution one can distinguish a strongly osmiophilic inner thread like the wick in a candle surrounded by a much less osmiophilic enwrapping mantle

On their surface all the above mentioned false chordae or trabeculae containing Purkinje's fibers are covered by the endocardium (endothelial cells) Inside as is well known they are very rich in sarcoplasm and poor in muscular elements The latter individual myofibrils are very similar to every other myofibril Their protofibrils are recognizable and the different zones such as Z M I H exhibit their usual significant differences The Z-bands which in the myocardium connect the individual myofibrils at the level of the Z-zones seem to be entirely missing in Purkinje's fibers In the heart muscle the Z bands are connected with the sarcolem but as yet I have not been able to find the typical garland type of sarcolem connected with Z-bands in these trabecules there is rather a straight double lined sarcolem

Very often one finds a single myofibril surrounded by a large amount of sarcoplasm and sometimes two or three myofibrils are found lying close together The familiar picture of masses of myofibrils lying parallel to each other separated by long rows of sarcosomes as can be seen in our different figures of the heart muscle does not exist here apparently One gets the impression that Purkinje's fibers in the false chordae simply do not contain normal myocardial muscle fibers of normal structure separated from each other by great amounts of sarcoplasm but rather that Purkinje's fibers contain within their sarcolem many individual myofibrils distributed within a great amount of sarcoplasm Such muscle fibers are concentrated in the central osmiophilic cylinder of the big false chordae and of the small endocardial trabecules of the heart The number of sarcosomes found between the myofibrils seems less than that present in the muscle fibers of the myocardium of the same animal They have a membrane and contain an inner laminar structure and also occasionally small vacuoles This smaller amount of sarcosomes in the conductive system of cattle may be as emphasized before connected with the observed fact that the conductive system is more easily exhausted under conditions of starvation or lack of oxygen than is the myocardium However not only the conductive system but the myocardium of cattle too contains less sarcosomes than that of small animals and that of man (see p 66) Most striking is the very large number of finest vesicles channels and lacunes of every size found

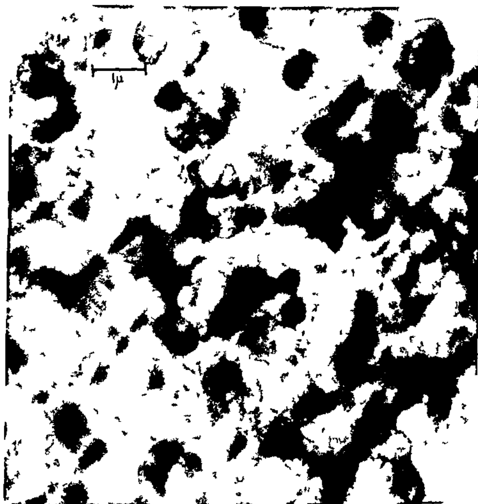


Figure 69 From the finest trabecules in the ventricle of a cow containing isolated myofibrils (Purkinje fibers) in a great amount of cytoplasm No Z bands can be seen but different sarcosomes and the Z zones of the myofibrils 12 000×

within the cytoplasm of Purkinje's fibers and between them This astonishing finding leads to the conclusion that this system of vacuoles and channels which may correspond to the endoplasmic reticulum in other cells is in some way involved in the great capacity for quick stimulus conduction of Purkinje's fibers Larger concentrations of plasmasomes (mitochondria) are often located just adjacent to the channels of this system In line with the fact emphasized above that the enzyme bearing plasmasomes are

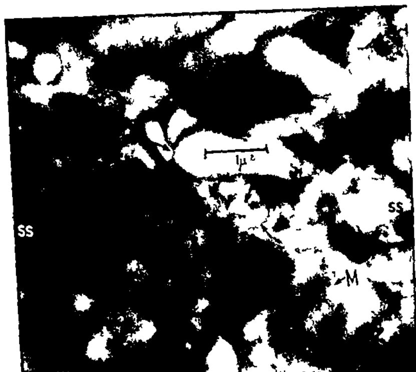


Figure 70 Purkinje fibers of the heart of a cow The sarcosomes (SS) are very scanty and the system of small channels (↑) in the cytoplasm abundantly developed Notice also like in the pervious figure the presence of the fibrilar zones Z M H I 16 000×

always aggregated near organelles with a high rate of metabolism such as the muscle fibrils in the myocardium or the nuclei in the heart and in the capillaries this accumulation of plasmasomes would speak in favor of a high metabolism in this system of channels That suggests again their having a significant function in stimulus conduction

From the viewpoint of the physiologist one should notice that there is no difference histologically between the myofibrils of the myocardium and the myofibrils of Purkinje s fibers Therefore it is not understandable why these fibrils should conduct a stimulus much quicker than do those of the myocardium but physiological research has proven that the stimulus is conducted much quicker in Purkinje s fibers than in the myocardium This suggests that it

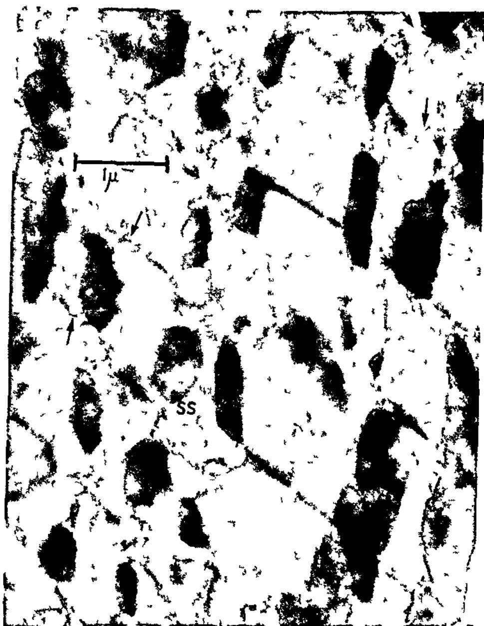


Figure 71 Questionable Purkinje fiber of rabbit (moderator band) Much cytoplasm between the individual myofibrils also sarcosomes also by arrows indicated vesicles 20 000×



Figure 72 A group of protoaxons surrounded by a Schwann's sheath from a very small trabecle containing Purkinje fibers in the cow's heart 18 000×

is the substance found in the muscle fiber between the myofibrils and not the myofibrils which determines speed of conductivity Perhaps it is also responsible for stimulus conduction in general In this regard there is a definite difference between the muscle fiber of the myocardium and that of the conductive tissue The cytoplasm between the myofibrils in the latter is much more abundant and the spaces of endoplasmic reticulum lacunes and cisterns of *liquid* are much bigger and more plentiful than in the myocardium (Figs 69 70 and 71) The concept that this part of Purkinje's fibers and the entire conductive system serves a specific function is well compatible with a similar opinion of H Rusca *et al* (1956) about the endoplasmic reticulum

Nuclei can be also found in Purkinje fibers and occasionally as seen in our figure one finds vessels which represent capillaries or finest lymph vessels Nerve axons are also present even in the smallest trabecules of the ventricles (see Fig 72)

It is well known from light microscopy that the trabeculae and the false chordae in which Purkinje's fibers are enclosed are extremely rich in lymphatic vessels It may well be that all the

mentioned very small vesicles, canals and lacunes or cisterns, which can be seen in Purkinje's fibers are the finest ramifications of lymphatic vessels and not part of an endoplasmic reticulum. Nowhere can one find the small particles attached to these canals which Palade found to be typical satellites of the endoplasmic reticulum (1958). Of course, where is the strict borderline between endoplasmic reticulum and the finest ramifications of lymphatic vessels? The location alone (intercellular vs. intracellular) is significant but still may not be decisive. In distinction to the protocapillaries (see next chapter), whose wall always consists of an endothelial cell, the profiles of the system of canals and lacunes in our pictures of Purkinje's fibers never show a surrounding endothelial cell but always an easily recognizable membrane only. The big lymphatic vessels outside the muscle fibers, however, are tubes built out of endothelial cells, similar to the capillaries.

In the moderator band of the rabbit we have found many muscle fibers exactly resembling normal myocardial fibers of the same animal, with great masses of sarcosomes. We have also found there another type of muscle fiber with isolated myofibrils embedded in a large amount of cytoplasm and fewer, though still many, sarcosomes.

It is known (Monckeberg 1908) that some of the spurious chordae of the heart contain only Purkinje's fibers, some only regular myocardial muscle fibers, and some contain both. The last seems to be the case in the moderator band of the rabbit. Intercalated discs of the same appearance as in the myocardium have been found in Purkinje fibers also.

In the chordae spuriae are found many capillaries and individual nerve axons as well as smallest nerve fibers. It is noteworthy that a structure like the rabbit's moderator band, traversing the right ventricle freely and having a diameter of only a fraction of a millimeter, is not supplied with blood only from the content of the right ventricle via the surface, but rather from innumerable capillaries as well. This factor points to the importance of an abundant blood supply to Purkinje fibers in the moderator band and to the physiological importance of its normal function.

XVI

THE CAPILLARIES

A NORMAL CAPILLARIES

Early in my investigations I had assumed on the basis of the electron microscopic pictures that some portion of the capillaries of cardiac muscle did not merely run between the muscle fibers but rather penetrated into these fibers this opinion had seemed to be borne out in the examination of stained light microscopic preparations With the development of electron microscopic technique and more especially in studying the ultramicroscopic teased tissue preparations we have recently been consistently able to demonstrate a fine membrane the sarcolem between the capillary wall and the muscle fibrils Assuming that this fine membrane always represents an actual muscle fiber boundary it would seem appropriate to assert at least until it can be proved otherwise that capillaries in the myocardium run in spaces between muscle fibers (1956b) and not within the muscle fiber proper

In the text that follows when we speak of the structure of the capillary wall we will have reference exclusively to the capillaries of heart (auricle as well as ventricle) and of skeletal muscle It is a well known fact that the capillaries of other organs such as the liver or the kidney exhibit unique peculiarities of structure (Krogh) Even the capillaries of the lung have been shown to possess certain peculiarities of structure and it should therefore be stressed that the capillaries under discussion in this section are only those found in muscle tissue

One can safely estimate the thickness of the wall of the most primitive capillaries at between 0 2 and 0 5 μ This is the case except in places where the sections contain a nucleus or the cytoplasmic cushion in which the nucleus is embedded At such places the wall of the capillary may have a diameter of 1 μ or more This fact can also be demonstrated by examining serial sections

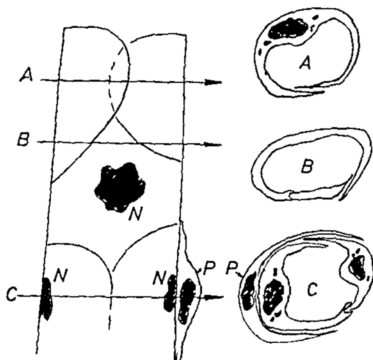


Figure 73 Diagrammatic representation of the simplest type of capillary (protocapillary) The three schematic section planes a b and c illustrate the various possibilities as to appearance in the electron microscopic picture which such a protocapillary can present *All sections are assumed to have passed directly perpendicular to the long axis of the capillary In oblique sections the possible appearances are of course much more varied still* N is the nucleus P is a pericyte (Rouget cell) surrounding the capillary tube with its cytoplasm

When they are not totally collapsed (Figs 84 and 85) the simplest capillaries viewed through the electron microscope exhibit one of three forms depending on the plane of sectioning The first form assumed by the capillary is that of a more or less perfect circle surrounding a lightly granulated surface The second form if sectioned obliquely is that of an ellipse and the third form seen when the plane of section cuts the capillary through its long axis is that of two ribbon shaped structures running parallel to one another The finely granulated area surrounded by the capillary wall is the space which in life is occupied by the blood as it flows through the capillary In this space one frequently

sees sections of red or white blood corpuscles (see Figs 73 78 79 etc) or thrombocytes

The simplest type of capillary is a tube consisting of a sequence of single endothelial cells only This I have called a protocapillary A section through such a capillary exhibits the clarity and simplicity of structure of the capillary wall It would seem incidentally that the thickness of this wall depends in part on the extent of filling of the capillary itself (A Krogh 1936 Kisch 1954c 1956b)

Two types of capillaries can be distinguished One which I call the protocapillary is a sequence of irregularly shaped endothelial cells with a widely expanded cytoplasm each of which at least in some places enwraps entirely the capillary blood stream Figure 73 shows in a diagram this situation and the electron microscopic appearance of different sections through the protocapillaries in the heart muscle auricles and ventricles Electron microscopy was able to reveal the enormous amount of such protocapillaries in the heart

Wherever the diameter of the capillary blood stream is too big to permit a single endothelial cell to embrace it completely two or more such cells will be needed to build the capillary tube The histological picture of the capillary wall will then present that of a mosaic of endothelial cells familiar from light microscopy In cross sections there never will be only one slit in the capillary wall but at least two usually even more and the cytoplasm of these capillaries is no longer so widely extended as in protocapillaries and this results in a thicker wall than in protocapillaries *In this* consideration there is mention made only of the endothelial cells

Pericites surround protocapillaries as well as capillaries with mosaic endothelium They become more frequent and smooth muscle cells *are added* when the pre or post capillary stage is reached

The capillary regularly exhibits an outer fairly regular membrane (membrana externa) The thickness of this membrane if double lined is approximately 300 Å It is the boundary between the capillary and the surrounding tissue and may be regarded as the basal membrane of the endothelial cell This simplest type of capillary is made up of a sequence of single endothelial cells

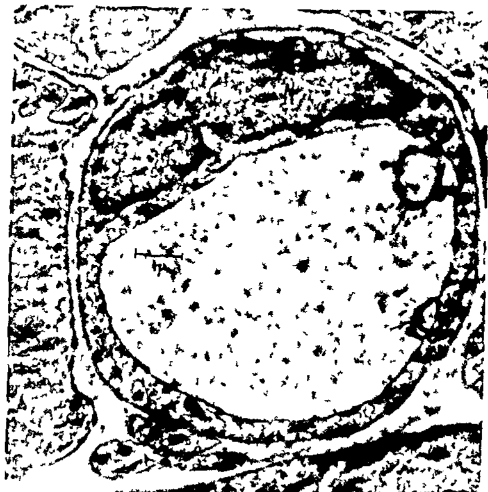


Figure 74 Protocapillary from the right auricle of a guinea pig An arrow indicates the slit in the capillary wall the other arrow the endothelial tentacles or villi The nucleus N plasmasomes near to it and profiles of finest channels in the cytoplasm can be seen 11 000×

adjacent to one another The line which separates the capillary *wall from the content of the capillary (membrana interna) always* is seen in electron microscope pictures as a single line This line does not run exactly parallel to the membrana externa but rather it pursues an irregular scalloped course The membrana interna exhibits also fine processes which extend like pseudopods or fringes *into the lumen of the capillary I called them the endo*thelial villi or tentacles of the capillaries

One is especially struck by these extremely fine long (in longi



Figure 76 Capillary of the human ventricle In the upper part the cytoplasm must be sectioned near the nucleus as suggested by its diameter in this place and the presence of plasmasomes (P) which are usually found near to the nucleus The arrow indicates the slit in the capillary V an endothelial tentacle PE part of a pericite (Rouget s cells) A a neighboring nerve axon The arrows in the cytoplasm hint to profiles of a system of fine channels (Endoplasmic reticulum) 12 000×

tudinal sections) thread like extensions reaching from the endothelial wall into the lumen of the capillary (see Figs 74 75 77) As sections sometimes cut these extensions through their entire length it might be more correct to regard them as thin ribbons rather than as hair like cilia It is only in longitudinal sections that these extensions appear as cilia In some pictures one sees

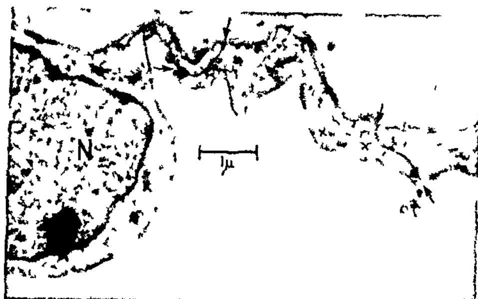


Figure 76 Longitudinal section through capillary in the ventricle of a mouse N endothelial nucleus The arrows point to two slits in the wall the crosses to profiles of a system of small channels in the cytoplasm No cement exists between the endothelial cells 13 000×

them as separated pieces lying within the capillary content cut off at their bases This appearance is illustrated in our Figure 83 designated by a star Such extensions are frequently found at the slits within the capillary wall that will be discussed later on They measure approximately 300 to 800 Å in diameter and they may be several microns in length Our Figures 74 and 75 show them in animal capillaries as well as in human ones

The two membranes discussed above the inner of which is more of the type of an interphase than a true membrane enclose between them the protoplasm of the endothelial cells and form together with this protoplasm the capillary wall This fact would lead one to suppose and indeed our figures bear this out that the capillary wall does not have a uniform thickness throughout It has, for example been mentioned that at the places where the nucleus together with its plasmasomes is found to lie between the membrana externa and the membrana interna the thickness of the capillary wall at that place where the nucleus has its maximum diameter, will approach 2 to 3 μ or even more In contrast in

areas far removed from the nucleus where there is very little protoplasm near the membrana externa the diameter of the capillary wall is found to be only about 300 Å

In this type of investigation one is again and again struck by the fact that the heart (auricle and ventricle and even the moderator band) contains a far greater quantity of capillaries than does the skeletal musculature of the same animal

The protoplasmic mass between the two bounding membranes of the capillary wall contains nuclei plasmasomes fine granules and vacuoles all in a uniform connecting medium The protoplasm also contains an ultramicroscopic system of canals similar to that which is designated as endoplasmic reticulum in the cytoplasm of other cells (Kisch 1957b 1957g Moore and Ruska 1957h) and many smallest droplets

From the physiologic point of view all of the above signifies that, thanks to the corrugated contour of the membrana interna with its extensions and tentacles the inner surface of the capillary wall must be far greater still than our present estimates (Krogh) would lead us to believe Perhaps this architecture of the inner surface of the capillary wall may contribute to a slowing down of the peripheral blood flow within the capillary Perhaps the long processes extending into the blood stream play some part in clot formation Our figures also point out that it is not correct to regard the capillary wall simply as an extremely thin permeable or semipermeable membrane Rather it is a complicated protoplasmic living system whose active selective function may play a key role in the permeability of the capillary wall Finally one can easily imagine (Kisch 1955a, b 1956b 1957g) that a thickening of the capillary wall under pathological conditions to twice its normal diameter a phenomenon which would completely elude the light microscopist would present an insurmountable barrier to the gas and other chemical exchanges which normally take place in the area of the capillary This thought poses a whole new series of problems and opens new vistas to the field of circulatory pathophysiology and so do the droplets in the capillary wall

In capillaries whose walls are made up of a single endothelial cell the boundary between their adjacent margins can present one of several appearances Most often the cells will overlap in the

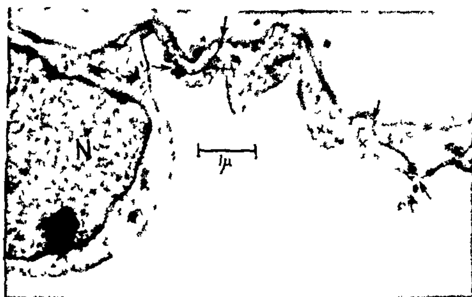


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Figure 78 Capillary from the human ventricle C Lumen of capillary Sl Sarcolem M Myofibril SS Sarcosome Many droplets in the capillary wall and at the inside of sarcolem Arrows indicate where droplets empty into intercellular space (Droplet transfer Droplet-dialysis) 40 000×

It has been known for a long time that capillaries whose walls are made up of a single endothelial cell layer are not constructed in such a manner that cells of cylindric form and parallel base lines are positioned with their borders touching one another to form a long cylindric tube Rather the cells are arranged in a spiral fashion (Benninghoff 1930) corresponding to their polygonal or rhomboid shape (Krogh) Because of this phenomenon a section transecting a capillary at an angle perpendicular to its long axis does not necessarily although it may produce a picture of a ring



Figure 77 A piece of a capillary from the human auricle showing the origin of one of the thread shaped extensions of the endothelial cells 46 000×

manner of a rolled up piece of paper leaving a diagonal slit between the borders (see diagram Fig 73) In other cases, the borders will fit tightly with close indentations or will intermesh to form a joint (dovetail or zigzag) (H Ruska 1954 Kisch 1955a b d, 1956d 1957g Palade 1956) In each of these cases the final result is a slit or a split which connects the inside of the capillary to the outside As yet *I have never found special material to be contained within these mural slits* The electron microscopic pictures seem to exclude the existence of any kind of cement within such slits (1957g) *These slits in the capillary wall could well serve to facilitate exchanges into and out of the capillary* One frequently finds the above discussed endo capillary extensions (endothelial villi) reaching into the capillary blood stream exactly at the site of these slits in the capillary wall

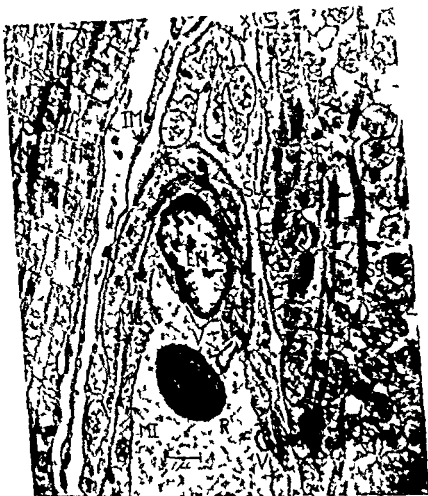


Figure 79 Capillary from the left ventricle of the guinea pig heart A pericyte (P) is seen to be sitting like a cap over the capillary in the region of the endothelial cell nucleus (EN) The structure directly above the pericyte (X) presents a group of nerve axons It contains as do similar pictures not published in this volume longitudinally and transversely sectioned fibrils of the finest diameter To the left there is seen an extension shaped like a membrane (IM) surrounding the capillary Its connection with the cell body of an interstitial cell can be seen above To the right and to the left of the capillary there is a muscle fiber whose myofibrils and sarcosomes can be easily identified 10 000×

composed of a single endothelial cell Although this type of structure is seen in some pictures, one frequently finds that the section has cut across two, or even three, endothelial cells Our diagrammatic Figure 73 illustrates the possible resultant configurations when a capillary constructed in the manner described above is sectioned in a plane perpendicular to its long axis As the schematic diagram shows the section can also contain either one or more nuclei In this connection, it should be kept in mind that these endothelial cells sometimes possess more than one nucleus (Benninghoff 1930) It is, of course also conceivable that the ultra thin section cuts through no nucleus at all This is illustrated in several of our pictures (Figs 75 and 80) If in such a section demonstrating no nucleus, one finds an area in which the capillary wall seems unusually thick (see Fig 75), perhaps even containing a few plasmasomes one can assume that the section passed in close proximity to the nucleus bringing into the section the cytoplasmic cushion in which the nucleus had resided, but missing the nucleus itself Generally, the nuclei bulge the membrana interna in toward the lumen of the capillary The nuclei can occasionally also push the capillary wall toward the outside *As our schematic diagram (Fig 73) illustrates* the capillary wall can exhibit one or more mural slits depending on the plane of sectioning It is only rarely that one sees no mural slits at all and when this occurs it is more probably the fault of a poorly focused photograph than the result of the absence of a mural slit

The above described ultramicroscopic structure of the capillary wall is found only in the simplest unicellular capillaries the protocapillaries and is a hallmark of this category of vessel The next step in the development of the capillary towards the pre capillary or post capillary is its widening to the mosaic like arrangement of endothelial cells around the blood stream There is further on an addition of certain cells surrounding the capillary tube which are collectively termed pericytes Rouget cells or satellite cells (Rouget 1873 1879) These cells are closely applied to the outer surface of the capillary wall Their nucleus in cross section is sometimes seen to sit like a cap over the nucleus of the endothelial cell (see Kisch 1956b Figs 5 and 6) The protoplasm of the pericyte surrounds the capillary like a hood without enclosing it



Figure 80 Capillary from the human ventricle between two muscle fibers and an interstitial cell with big nucleus (N) V endothelial villi A Two nerve axons in contact with the capillary SL Sarcolem of the muscle fiber SS Sarcosomes The thick part of the endothelial cytoplasm is a part near to its nucleus

completely, and nowhere does the protoplasm of the pericyte intermingle with that of the endothelial cells Our pictures illustrate various sections which show how the endothelial cell of the capillary is surrounded by one or more Rouget cells in whose protoplasm one can repeatedly identify nucleus and plasmasomes By means of the electron microscope, we can differentiate the membrana externa of the capillary clearly from the cell wall of the pericyte, a statement which was understandably not so easy for light microscopy Several pericytes occasionally overlap one another, or surround the capillary from different directions their protoplasm enclosing the capillary like a sheath of surrounding tissue (Figs 79 and 80)

In the part of the vascular system which is known as the pre capillary and postcapillary vessels, the diameter of the tube increases in size, and the endothelial layer of these vessels consists then, in each section not of one or two single endothelial cells, but of a larger number As a rule the typical picture of the protocapillaries then disappears The individual endothelial cells are no longer flat thin layers of cytoplasm but the cytoplasm tightly surrounds the endothelial nucleus in globular form In this way the endothelial layer of such small vessels presents the picture known from light microscopy as being a mosaic of endothelial cells surrounded by layers of other cells and finally, further away from the protocapillaries also surrounded by smooth muscle cells

It should be emphasized that in these pre and post capillaries I also have never seen any material intercalated between the individual endothelial cells which could be interpreted as the cement familiar to light microscopists I have emphasized for many years that *I strongly doubt the existence of such a cement* which may either be an optical artifact in light microscopy or a precipitation due to the processing of the material for light microscopic investigation

The result of our own investigation of the vessels concerning the border lines of endothelial cells must therefore be *a connecting cement between endothelial cells does not exist in the capillaries, nor in other vessels investigated by us to date* The opinion has been differently proffered that in scurvy the bleeding into the tissue is due to any abnormality *of the connecting cement between*



Figure 82 Capillary from the leg muscle of an albino mouse Two endothelial nuclei are visible as are endothelial cell extensions The capillary is surrounded by two Rouget cells whose plasmasomes are visible 11 000×

such experimentations are at present in progress It is tempting to suppose that red or white blood cells in diapedesis do not figuratively speaking drive with their heads into the wall when an open door is available to them Of course however such speculations are of little value without the microscopic proof to support them

Ever since Rouget discovered the pericytes the literature concerning these cells and their physiological significance especially

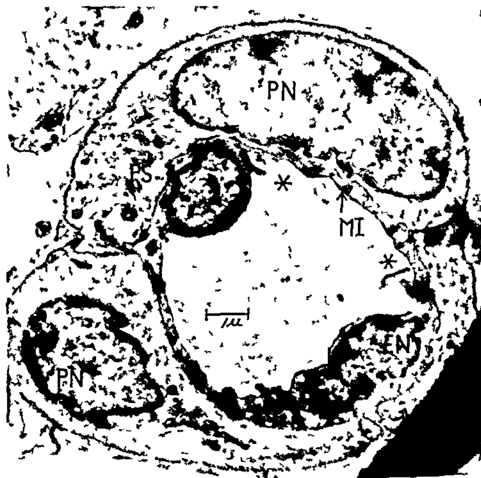


Figure 81 Capillary from a leg muscle of the same mouse like Fig 82 Most probably another section through the same capillary as that illustrated in Figure 82 The nuclei of the pericytes (PN) surrounding the capillary are clearly visible in this picture 10 000×

the endothelial cells of the capillaries giving the blood cells free space to leave the intracapillar blood stream In our opinion such a theory must also be rejected first because even normally no such cement exists second because this author in studying the vessels of animals with severe scurvy electron microscopically has never found conditions that would justify such an assumption

It is at present impossible to say what physiological and pathophysiological significance the mural slits of the capillary may have Further experimentation will be required to determine this and



Figure 84 A totally collapsed capillary Within the capillary wall various plasmasomes and extremely fine canals sectioned longitudinally and transversely are visible Note an elongated thread like endothelial extension in the lumen of the lower portion of the capillary (↑) The illustrated capillary is from the left ventricle of guinea pig heart 22 000×



Figure 83 Totally collapsed capillary from the heart of a guinea pig Within the lumen whose diameter at the widest part is approximately *one half a micron* many elongated endothelial cell extensions are visible 20 000×

their possible connection with vascular contractility has been extensive indeed (see Krogh 1936) At present our pictures do not permit any conclusions on this point The obvious folding of the membrana externa in our Figures 81 and 82 would of course, be compatible with a theory that the state of tension or contraction of the pericytes seen surrounding these vessels is the causative factor of the observed state of the capillary wall

B THE PICTURE OF THE TOTALLY COLLAPSED CAPILLARY

Up to this point the capillaries which we have shown in our illustrations have all been more or less filled by material in their lumen although these capillaries under the light microscope might well have appeared as collapsed Some of our capillaries even contain red or white blood corpuscles The filiform extensions which we noted running from the membrana interna into the lumen of the capillary give an appearance of being suspended in a fluid medium In cross section these extensions often had a curlicue appearance (see Figs 75 77 79)

The picture of the totally collapsed capillary is very different Here the membrana interna in longitudinal sections is separated

C ABNORMAL CAPILLARIES

It was mentioned above that the probably abnormal configuration of the capillary wall under pathological conditions may be of high and as yet not appreciated importance The exchange of material between blood stream and tissue cells is thus affected and consequently tissue metabolism at large may be vitally affected as well In this regard due to the severe handicap of our work as mentioned in the introduction our own experiences are still very limited and mainly involve two pathological conditions One concerns acute pulmonary edema which we have produced in rabbits by intravenous injection of suprarenin The animals were killed when in dying condition with pulmonary edema fully developed or when the symptoms of pulmonary edema had just started to develop The capillaries of the lungs were then investigated (1958a)

As our pictures show our main findings were that many of these capillaries showed a thickening of the endothelial as well as of the epithelial cytoplasm The endothelium became swollen contained vacuoles and displayed large blisters Finally it dissolved entirely Similar changes were found in the epithelial layer of the capillaries of the lung

These findings correspond closely to those of Meessen and Schulz (1959) based on other experimental methods and published shortly prior to our own findings In acute pulmonary edema as shown by us (1958a) the endothelial cells in the smallest airducts of the lung produce abundant droplets which must also contribute to fill these air spaces with fluid These findings lead to considerations as to whether the capillaries of the lung are really the only capillaries suffering under conditions leading to acute pulmonary edema Indeed this does not seem to be the case Under these conditions for example we found a thickening of the endothelial wall in some of the capillaries of the heart and we have noticed similar changes in scorbutic guinea pigs in experiments performed together with Dr G J Martin I think that in the future the concept of a unified pathological condition a generalized *capillary edema* should be kept in mind if it can be verified by further experimentation

from the internal boundary of the opposite side by only the finest separation, measuring approximately 500 to 5 000 Å Sometimes the membrana interna of one side actually touches the membrana interna of the other side however it is always possible to recognize the existence of two distinct membrane layers in such a case The scallopped nature of the surface is, in general, not obliterated, and the very fine tentacles noted above, whose diameter is approximately 300 to 800 Å do not disappear, but are found to be neatly stretched out and packed into the narrow space which represents the collapsed lumen of the capillary All this is illustrated clearly in our Figures 83 and 84 which show collapsed capillaries from the guinea pig heart (left ventricle)

If a cross section through a totally collapsed capillary passes directly through an endothelial nucleus the picture may be somewhat different Here as elsewhere the slit which represents the lumen of the capillary is ultramicroscopically small Despite this however one can easily see that the nucleus projects far into the lumen of the capillary so that the tiny space which represents the interior of the capillary comes to assume the form of a Y or of a tiny slit Seeing such pictures one can conceive that in the case of total collapse of a capillary the nucleus projecting as it does into the lumen contributes to the total obliteration of the space

In sections passing through the long axis of a capillary or in diagonal sections one can see that total collapse such as described above may occasionally occur in only a portion of the vessel so that a totally collapsed portion of capillary may be bounded by another portion whose lumen is patent and as seen in some of our sections even contains some blood corpuscles Thus while a capillary may be totally closed to the blood stream in reality the section of the vessel whose lumen is actually closed to the point of disappearance may be only several microns in length In adjacent though patent sections of the capillary the blood stream is stopped so that blood corpuscles amass in the stagnant liquid

The author s own research has not yet advanced sufficiently to allow for an exact description of the ultrastructure of the more complicated components of the circulatory system such as precapillaries, arterioles etc It is our hope however that in the not too distant future we will be able to discuss these subjects as well



Figure 85 Endothelium from the atrium of a normal guinea pig A big nucleus (N) in the endothelial cell is seen In the sub-endothelial stratum which separates the endothelial cells from the sarcolem (SL) a nerve axon (A) 18 000×

Other structures of the heart richly supplied with nerves are the capillaries both of the atria and of the ventricles The nerves here are again single axons which make direct contact with the capillary wall Rarely two or more such single axons are found close to each other and in contact with the capillary wall Their diameter is between 0 5 and 2 microns They have a membrane are of circular or elliptic shape and sometimes produce a tapering extension along the capillary wall They contain as a rule cylindrical

XVII

INVESTIGATIONS ON CARDIAC NERVES AND THE MECHANISM OF PAIN IN ANGINA PECTORIS*

Light microscopy and physiology have provided us with many basic facts concerning the cardiac nerves. It is common knowledge that the heart is well supplied with nerves of the autonomic system (sympathetic and parasympathetic nerves). In certain parts of the heart, such as the bundle of His or the sinus node, this has been demonstrated best. It is also known that centripetal nerves are involved in reflexes of the heart, such as the Bainbridge reflex or the Bezold-Jarisch reflex. Centripetal nerves of the heart must also be involved in the painful sensation of angina pectoris.

Previously (1956a, 1957b) attention was called to single axons which can be found to exist in great numbers in a homogenous subendothelial stratum that spreads between the endocardial cells and the sarcolemm of neighboring muscle fibers in the auricles as well as in the ventricles. Most of these axons are 0.3 to 0.5 μ in diameter and are surrounded by a membrane. They contain one or more oblong bodies of cylindrical form up to 2000 Å in diameter and also little dots and fibrils 200 Å or less in diameter, which represent internal structures. To date, we have found no nerves between the endothelial cells proper.

The existence of the subendothelial stratum and its content of axons has recently been confirmed for the atrium of the turtle as well (Fawcett and Selby, 1958).

* Part of this and the following Chapter of the book and the different pictures used therein have previously been published in the journal *Experimental Medicine and Surgery*. They are reproduced here with the kind permission of its publisher, Brooklyn Medical Press, New York.



Figure 87 Guinea pig Part of a capillary wall (C) with an adjacent nerve axon (A) which contains some big osmiophilic particles Two of them exhibit an inner lamellar structure Different small vesicles are seen in the axon (endoplasmic reticulum or droplets) (R) Red cells 40 000×

bodies and occasionally finest fibrils similar to those seen in the axons of the subendothelial stratum of the heart The bigger (1000 to 2000 Å diameter) bodies are probably plasmasomes (mitochondria) Some of them even have a visible internal lamellar structure The fact that in ultra thin sections of the heart many of the capillaries exhibit nerves at their periphery is proof of the enormous number of axons present at the capillaries of this organ This constitutes a very definite difference between the capillaries of the heart and those of the lungs which have been examined previously

Besides the numerous axons attached directly to the capillaries there are also other axons of different diameters occurring singly

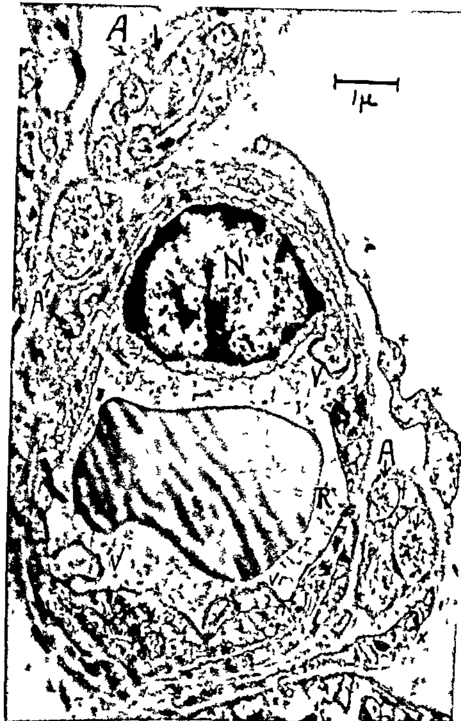


Figure 86 Guinea pig Capillary in the left ventricle (N) Endothelial nucleus (V) endothelial villi (A) two groups of axons and a single one (A) Notice the difference in the diameter of the individual axons Arrows indicate very fine fibrils in the protoaxons some sectioned longitudinally some transverse (R) Red blood cell 15 000×

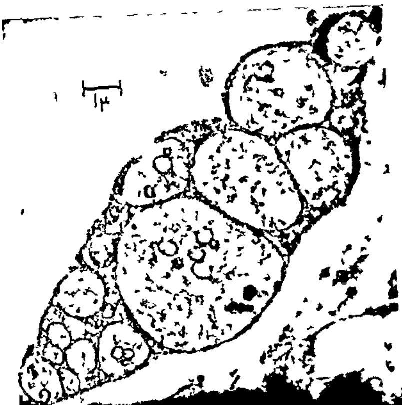


Figure 88 Small nerve fibril from the left ventricle of a normal rabbit 18 cross sectioned protoaxons are surrounded by a Schwann's sheath Notice the very different diameter of the axons They contain osmiophilic bodies and small vesicles Between the axons is some cytoplasmic material consisting of lighter and darker droplets and vesicles 10 000×

suggests two conclusions the one concerns the sensibility of the capillaries of the heart in contrast to those of other organs especially the lungs while the other concerns the structure of small nerve fibers and consecutive physiologic problems (See the next chapter)

Of course the lungs like the heart are well supplied with nerves However the capillaries of the lungs are not supplied with nerves whereas as our pictures show there is an abundant amount of nerves in close proximity to the capillaries in the atria as well as in the ventricles of the heart many of them in direct

and in bundles. They can be found near the pericytes (Rouget cells) and between the individual muscle fibers of the heart. They probably supply both the Rouget cells and the fibers, but they have never been found inside the muscle fibers between the myofibrils. No specific nerve endings at the muscle fibers of the heart have yet been found. See Fig. 88 and Chapter XIX.

The most outstanding feature of these groups of axons found near the capillaries and between the muscle fibers of the heart is the fact that the axons are very different both in diameter and in their contents. Some of them show the above-mentioned big cylindric enclosures, and others show instead very thin fibers which appear in cross sections as little dots and in oblique sections as very fine parallel lines. Some axons show both of these structures. From these groups, which are surrounded by the sheath of a Schwann cell, the single axons apparently separate to reach their final destination, the capillaries or the muscle fibers.

The internal structure and size (0.5 to 2 μ diameter) of the individual axons in the sub-endothelial stratum is very similar to that of most of the axons in contact with the capillaries. We have as yet no means whereby to differentiate the different nerve axons (afferent and efferent) according to their appearance. All the axons found in the heart so far are of the non-myelinated type.

A clue to differentiating nerves may be given by the fact that the axons in the sub-endothelial stratum are all of a similar type, the diameter being 0.5 to 2 μ, and that they usually contain cylindrical bodies (or circular cross sections of them) with a diameter of ca. 1500 to 2000 Å. They also contain fine fibrils with a diameter of 200 Å or less. The endothelium of the heart is not, to our knowledge, supplied by centrifugal nerves. However, influences on the endothelium are known to start reflexes such as those described by Francois Frank, Bezold, Jarisch and others. This proves the presence of centripetal nerve fibers. Probably the fibers are those found in the sub-endothelial stratum.

The capillaries are reached by single naked axons which are in direct contact with the capillary wall, as can be seen from our pictures. They sometimes show a tail-like extension along the capillary wall. These contacting axons are of a type very similar to that found in the sub-endothelial stratum. This observation

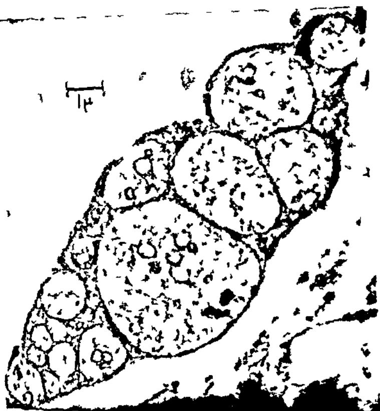


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contact with the capillary wall It seems very probable that this fact is responsible for the intense pain in conditions of inadequate blood supply to the heart as for instance in attacks of coronary spasm (leading to the pain called angina pectoris) or after occlusion, even of a relatively small coronary vessel By contrast major destructions of the lungs even such secondary to inadequate blood supply may occur without any associated acute pain

Concerning the internal structure of the small nerve fibers in the heart, our pictures show that nerve bundles of a diameter of only a few microns, scarcely visible to light microscopy contain many, distinctly separated individual axons of variable diameter Such entire groups being bundles of protoaxons are usually enwrapped by the sheath of a Schwann cell From this observation it can be concluded that in separating from a nerve fibril, the finest axons do not follow a pattern of arborization in which one bigger axon fiber splits in two smaller branches Rather, within any nerve fiber there are many individual axons (proto axons), all separated from one another (1955c) These axons probably run uninterrupted from the central cell to the periphery Their branching from the nerve fiber is in reality only a separating of individual units from each other It is not a division of one common fiber I also have never seen the dividing of one axon into two in diagonally sectioned axons or in longitudinal sections The recognition of this structure of the nerve fibers creates new problems for the physiologist and for the pathologist too For instance the usually accepted concept of the axonreflexes is incompatible with these histological facts (See Fig 85 and next chapter)

The individual most primitive axons or proto axons have a very different diameter Various reasons may be responsible for such a phenomenon Perhaps within a nerve fibril, axons of different diameter and cylindrical appearance exist next to each other as was proven to be the case by Tasaki and his collaborators (1944) and G Rudolph (1956) in myelinated nerves Recently similar observations were made in the atrium of the turtle by Fawcett and Selby (1958) The single axon is not cylindrical but changes its width irregularly according to its content of plasmasomes (mitochondria) (Kisch 1958c) (See Fig 86 and Chapter XIX)

It may also be that the appearance of the cross sections of axons in the heart should be attributed to the presence of different kinds of nerves for instance centripetal ones and centrifugal ones In this case I would be inclined for reasons mentioned above to regard the big axons with cylindrical and spherical osmiophilic inclusions as centripetal nerves Further investigations will be needed to clarify this point

The clinical syndrome of angina pectoris is well known for a long time Our knowledge concerning the mechanism of the severe pain observed in acute ischemia of the heart muscle is very vague however This ischemic pain is present in any type of coronary insufficiency in coronary occlusion in attacks of severe tachycardia and in tight aortic stenosis The usual explanation that sensory nerves become irritated under such conditions and cause pain is of course not satisfactory because sensory nerves are irritated in any kind of pain We know for example that similar deficiency of blood supply to a portion of the lungs does not produce similar intense pain despite the fact that the lungs too are well supplied with nerves Even the pain following multiple embolism to the lungs is hardly comparable in intensity to the pain of angina pectoris

The extremely rich nerve supply of the capillaries in the human heart has been noticed (1958c) and some evidence has been offered that these capillaries are supplied by centripetal and centrifugal nerve axons mainly by the former In electron microscopic studies on the ultrastructure of the circulatory system (1957g) ultra thin sections of the heart muscle (atria as well as ventricles) very frequently showed nerve axons in close proximity to or in direct contact with the capillary wall Considering this fact one can visualize the incredibly rich nerve supply of these capillaries By contrast this author's own previous investigations of the lungs and their capillaries established the fact that the capillaries of the lungs have no nerve axons in contact with them

These observations lead one to the conclusion that of the many nerves which supply the lungs as well as the heart those in contact with the capillaries are chiefly responsible for the pain of angina pectoris In spasm or occlusion of a coronary artery sudden chemical and physico chemical changes of the blood in the capillaries

contact with the capillary wall. It seems very probable that this fact is responsible for the intense pain in conditions of inadequate blood supply to the heart as for instance in attacks of coronary spasm (leading to the pain called angina pectoris) or after occlusion, even of a relatively small coronary vessel. By contrast major destructions of the lungs, even such secondary to inadequate blood supply may occur without any associated acute pain.

Concerning the internal structure of the small nerve fibers in the heart, our pictures show that nerve bundles of a diameter of only a few microns, scarcely visible to light microscopy, contain many, distinctly separated individual axons of variable diameter. Such entire groups, being bundles of protoaxons are usually enwrapped by the sheath of a Schwann cell. From this observation it can be concluded that, in separating from a nerve fibril the finest axons do not follow a pattern of arborization in which one bigger axon fiber splits in two smaller branches. Rather, within any nerve fiber, there are many individual axons (proto axons), all separated from one another (1958c). These axons probably run uninterrupted from the central cell to the periphery. Their branching from the nerve fiber is in reality only a separating of individual units from each other. It is not a division of one common fiber. I also have never seen the dividing of one axon into two in diagonally sectioned axons or in longitudinal sections. The recognition of this structure of the nerve fibers creates new problems for the physiologist and for the pathologist too. For instance the usually accepted concept of the "axonreflexes" is incompatible with these histological facts. (See Fig. 86 and next chapter.)

The individual most primitive axons or proto axons have a very different diameter. Various reasons may be responsible for such a phenomenon. Perhaps within a nerve fibril axons of different diameter and cylindrical appearance exist next to each other as was proven to be the case by Tasaki and his collaborators (1944) and G. Rudolph (1956) in myelinated nerves. Recently similar observations were made in the atrium of the turtle by Fawcett and Selby (1958). The single axon is not cylindrical but changes its width irregularly according to its content of plasmasomes (mitochondria) (Kisch 1958c). (See Fig. 86 and Chapter XIX.)

XVIII

THE MECHANISM OF AXON REFLEXES

The experience which this author has gained in investigating the nerves of the heart by means of electron microscopy have led him to certain conclusions concerning axon reflexes These will be proffered here because a great deal of the literature on axon reflexes concerns the cardiovascular system This is true for the investigations of Ninian Bruce (1913) on vasodilator axon reflexes and for the investigations on similar reflexes reported by A Krogh Sir Thomas Lewis and others Because recently there has been doubt as to the existence of underlying axon reflexes for some of these observed reflex reactions (Schaefer 1952) it seems necessary here to confront the physiological aspects with the histological facts

According to the 21st Edition of the *American Illustrated Medical Dictionary* (1948) an axon reflex is "a reflex resulting from a stimulus applied to one branch of a nerve which sets up an impulse that moves centrally to the point of division of the nerve where it is reflected down the other branch to the effector organ"

This definition expresses well what one usually has in mind when speaking about axon reflexes

It was probably the physiologist and biochemist Willie Kuhne who in 1859 first described an axon reflex fitting the above mentioned definition though he did not yet use the expression "axon reflex" At the centenary of Kuhne's discovery of 1859 it *seems* appropriate to check and revise our ideas concerning the mechanism of axon reflexes in the light of electronmicroscopic experiences

Kuhne's basic experiment his famous *Zwei Zipfel Versuch* (twotips experiment) was performed with the musculus sartorius of the frog

may be the irritant which stimulates the sensory nerves of these capillaries. Similar changes in the immediate surroundings of these capillaries due to an abnormal circulation may have the same effect on the centripetal nerves in contact with the capillaries. Such a concept could for instance explain the following well known fact. In an anoxemia test, the lungs as well as the heart are insufficiently supplied with oxygen. There is no pain in the lungs, even in high degrees of anoxemia, but there is pressure and pain precordially especially when a coronary insufficiency is present. This pain is also seen without a coronary involvement in cases of generalized anoxemia of the heart as, for instance, in a severe tachycardia or in aortic stenosis. We also know from experiments of E. Zak that if a tourniquet is put around one arm of a healthy person and he then does the same amount of exercise with both arms, he will soon feel severe pain in the arm with insufficient blood supply. The pain experienced by patients with intermittent claudication is also well known. Electron microscopic studies of skeletal muscle reveal that indeed the capillaries of this type of tissue also are well supplied with nerve axons (our observations).

This author's findings concerning the abundant nerve supply to the capillaries of the heart (1957g, see Figs. 60 and 69) have recently been corroborated by Meessen (1959). Axons adjacent to muscle fibers of the ventricle are much rarer but they too have been observed (Kisch, not yet published).

with some also on the pilomotors of the dog and on vasomotor reflexes of the skin of both these animals His conclusion was that his observations may also be applied to all classes of efferent nerves existing in the sympathetic system (p 364)

A Ninian Bruce reported in 1913 on *Vaso Dilator Axon Reflexes* summarizing in the introduction all the previous literature on the topic

Axon reflexes especially some involving the vessels of the skin were reported or at least suggested by many authors Among these were Th Lewis Krogh L R Muller Ebbecke Fleisch R Tigerstedt summarized most of the pertaining work up to 1923 in his classic *Physiology of Circulation* Recently however Hans Schaefer (1952) has uttered some doubt concerning the real existence of some of the axon reflexes described in the literature

Not many of the authors reporting axon reflexes have cared to give their opinion on the finer mechanism of such a reflex Most of these if not all seem to have in mind a mechanism as suggested by Willie Kuhne in Figure 90 We should like to call this the classic concept of axon reflexes A single nerve fiber divides itself into two branches Within this unit the impulse travels up one branch (centripetally) to the point of division and from there down the other branch (centrifugally) and probably also up the undivided fiber Ninian Bruce expresses his idea of the mechanism of an axon reflex as follows (p 351)

When the fiber comes near to the periphery it must bifurcate like the letter Y one limb passing to the sensory end organs etc in the skin and along this afferent impulses will pass while the other will end in the bloodvessels and along this efferent impulses will pass"

Such a concept is made of course under the never proven assumption that a primitive nerve fiber is able to bifurcate in the periphery like the letter Y It seems that nobody ever has had a serious doubt about this prerequisite of the classical concept of axon reflexes

In studying for many years the electron microscopy of the constituents of the circulatory system I have recently mainly investigated the cardiac nerves The result of these studies gave reason



Figure 89 Ventricle of the heart of the rat Next to capillary C with endothelial Nucleus N is a fine nerve fiber cross sectioned containing twelve protoaxons 8 000×

Our Figure 90 is a reproduction of Figure 9 in Kuhne s book of 1860 (p 98) The sartorius is cut at its tip with scissors to a point where there is finally an upper common part of the muscle and two separate tips (A and B) as in our Figure 90 A as well as B are supplied by one branch respectively of the nerve N which as can be seen, divides itself at point t into two branches These branches do not reach further than to line M The muscle beneath this line is bare of any nerve supply That just makes the frog s sartorius fitted for Kuhne s experiment He observed that chemical or mechanical stimuli applied to the nerveless part of tip B, f i at the level of a a a produce a contraction of the fiber B only A remains at rest If a stimulus is applied to the branch of the nerve in B at the level of line b (Fig 90) both tips of the muscle (A and B) contract Kuhne offered for this phenomenon the explanation which remained until today the concept of an axon reflex as indicated by arrows in his figure The impulse produced by the stimulus in b goes according to Kuhne centripetally within the nerve up to point t and from there centrifugally down into branch A Kuhne regarded this experiment as a convincing proof of the ability of motor nerve fibers to conduct impulses in both directions (*Doppelsinniges Leitungsvermogen der motorischen Nerven*)

It was in 1900 that J N Langley described exactly the same process for preganglionic fibers of the sympathetic system without reference to Kuhne s experiment Langley created for these pseudoreflexes the name axon reflex This name has been generally accepted ever since in neurology Langley s experiments were mainly performed on the pilomotor nerves of the cat

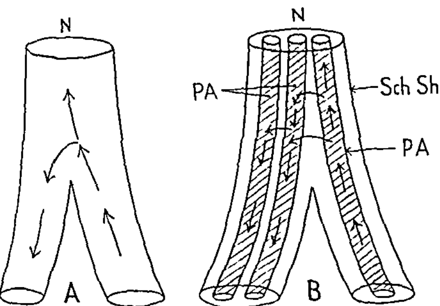


Figure 91 Diagram of an axon reflex A The classical concept B The new concept based on the electron microscopic pictures See text

tions are in best agreement with pictures of nerve tissue presented by other electronmicroscopists as for instance the fine work of H Gansler and others These authors however abstained from discussing the ultramicroscopic facts in regard to the branching of nerve fibers or the important repercussions of the electronmicroscopic findings on physiological problems

Our Figure 91 shows in form of diagrams in A Kuhne s concept of axon reflexes and in B the histological facts and the only possible mechanism for axon reflexes *if such reflexes really exist*

It seems remarkable indeed that the very same problems which we have just emphasized with regard to the electronmicroscopic structure of protoaxons and the dividing of nerve fibers were discussed already more than a hundred years ago At that time the discussion involved the smallest nerve fibers visible to the naked eye or those visible through the very primitive miscroscopes of this time Reil then regarded the thinnest nerve fibers to have a diameter of a hair of the scalp whereas other scientists thought such a guess to be wrong (H E Weber 1830) They supposed

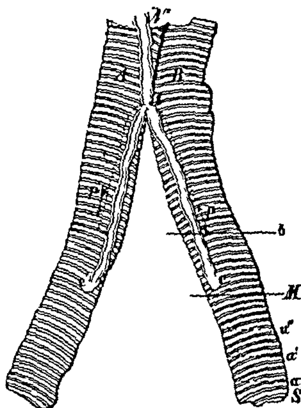


Figure 90. *Willie Kuhnes* (1859) drawing to explain the mechanism of his two tips experiment. See explanation in the text.

seriously to doubt whether branching and dividing of an individual most primitive nerve fiber really exists. I was able to prove for the unmyelinated nerve fibers of the heart and in not yet published investigations for the myelinated nerve fibers of the leg muscles the following fact. The smallest nerve fibers exist either as single axons (diameter 0.3 to ca. 3 μ) or as bundles of such *protoaxons* surrounded by a Schwann sheath. In diagonal or longitudinal sections I have not yet seen the dividing of a protoaxon. I came to the conclusion that according to electron microscopic evidence all branching of the finest nerve fibers is only a separating of preformed individual protoaxons or groups of protoaxons, never a dividing of a common stem similar to the stem of a tree dividing into branches.

If this new aspect proves to be correct, the classic concept of axon reflexes is untenable. Our own electronmicroscopic observa-

XIX

SOME PICTURES FROM THE HUMAN HEART

The material for the following pictures was taken from patients who died in a condition of chronic heart failure Particles from the ventricle of the heart were taken immediately after the death of the patient by needle biopsy method by Dr Mehmed Cavusoglu of the medical division of City Hospital at Elmhurst New York

Special attention is called to the many sarcosomes with disintegrated internal structure (osmiophilic degeneration) in these hearts Such sarcosomes are not found in the hearts of normal animals (See Kisch Cavusoglu and Marangoni 1959)

that the diameter of the smallest nerve fibers is one quarter of a hair diameter only Robert Remak reports a remark of his master Johannes Muller, that probably the so called finest nerve fibers are by no means really the finest ones and that the really finest ones are surely not yet known at that time (ca 1835) In this respect a remark of the great anatomist and physiologist H E Weber (1830) about ramification of nerves made in 1830 is noteworthy In literal translation it reads on page 277 of his book Most nerves divide approximately like a tree into smaller and still smaller branches but in most cases one can prove that all these branches starting from one nerve consist of fascicles of nerves or nerve fibers which already before the division were isolated within the stem and have had their own individual enwrapping, wherever this can not be proved the reason is the smallness of the branches However it has never yet been observed by anyone that a simple nerve fiber has divided into branches

This statement of Weber holds according to our experience for the protoaxons too They are probably or better surely just those finest nerve fibers (diameter from up to 0 3 μ) of which Johannes Muller and Robert Remak dreamed 120 years ago (Kisch 1954)

As mentioned above the physiological consequences of this recognition make it necessary to abolish the classical concept of the mechanism of axon reflexes as conceived by Kuhne (Fig 91A) If axon reflexes really do exist their mechanism ought to work according to the diagram in Figure 91B That of course creates a new very great difficulty an impulse ought to be transmitted from one protoaxon across its own axon membrane transversally to a neighboring one across the membrane of this second protoaxon and through the matrix that separates the protoaxons A biochemical stimulus transmission seems to fit such a setup much better than an electric transmission

That means that in case of axon reflexes we would have to assume that at least all the protoaxons enwrapped by the same Schwann s sheath are only anatomically but not functionally units isolated from each other

Such an aspect of course calls for a host of new experiments in the field of neurophysiology and applied biochemistry



Figure 93 Another picture of the human ventricle Notice the large amount of sarcosomes between the double lined sarcolem (Sl) and the myofibrils and between the individual myofibrils Some of the sarcosomes show an inner lamellar structure the content of others is retracted and disorganized 14 000×

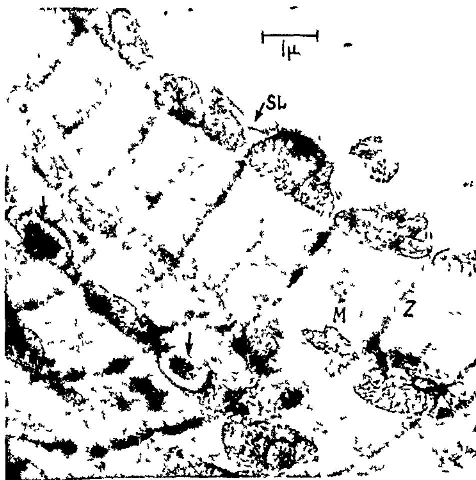


Figure 92 Myofibrils with typical striation The zones Z and M are clearly visible sarcosomes with an inner lamellar structure fill the space between the sarcolem (SL) and the myofibrils and the space between the individual myofibrils Two sarcosomes indicated by arrows show osmiophylic degeneration Their membrane is still present but their content is retracted and dark Length of each sarcomere (the distance between two Z lines) is about 2 microns Human ventricle 12 000×



Figure 95 Arrows point to an intercalated disc in the human heart A double lined zig zag membrane can be seen *The intercalated discs are regarded by most authors as boundary lines of the muscle cells of the heart* Notice the many sarcosomes (SS) some *of which* are in *osmiophylic de*generation 17 000×

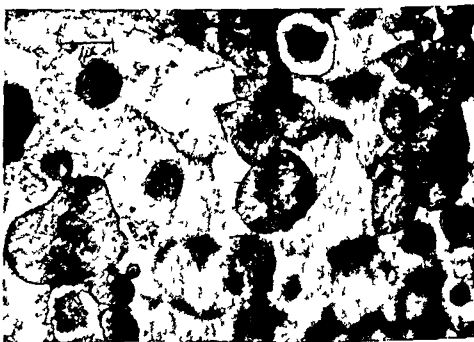


Figure 94 Sarcosomes of the human heart Some with normal inner structures two in osmiophylic degeneration Sarcosome indicated by arrow is very big and its content is a more or less homogenous material Next to it indicated by asterisk an osmiophylic body 12 000×



Figure 97 ER-cell of the human heart. Usually located near a capillary but also found between muscle fibres. Characterized mainly by the widened endoplasmic reticulum (ER) spaces, by many ghosts of plasmasomes (P) (mitochondria) and by many small granules in the cytoplasm. The nucleus (N) is usually eccentric. These cells are reminiscent of certain types of plasma cells. (Kisch 1960.) 12 000×



Figure 96 A capillary wall from the human heart C indicates the lumen of the capillary EN = nucleus of bizarre form of an endothelial cell In the cytoplasm of the cell are mitochondria and smallest droplets Attached to the external membrane of the capillary is a Pericyte PN = nucleus of the Pericyte Ax = nerve axons RE = part of an RE cell (see next pictures) Arrow points to slit in the capillary wall 8 000×

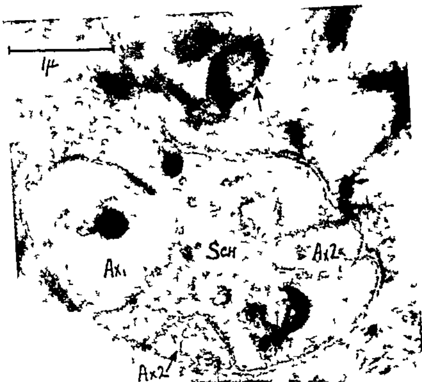


Figure 99 Section of a small nerve fibre between muscle fibres Three axons (Ax) are surrounded by a Schwann (Sch) cell Notice the difference between the diameter of the big axon Ax1 and that of two small axons Ax2 They probably represent two different types of nerves It seems that the axons are free of the Schwann cell at one side The body indicated by arrow may be a naked axon Mitochondria can be seen in the Schwann cell as well as in the axons and also in the smallest vesicles Outside of the axon finest fibrils probably collogen fibres can be seen 31 000×

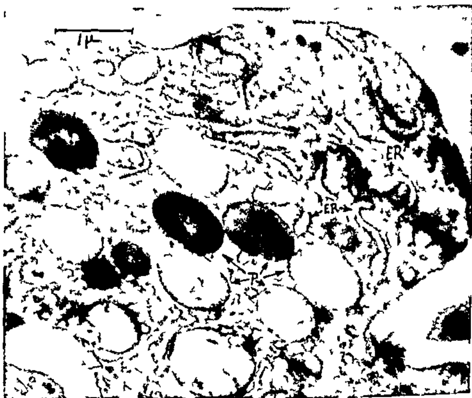


Figure 98 Enlarged part of an ER cell of the human heart Plasmasomes (mitochondria) with their dark content can be seen There are also ghosts of plasmasomes (P) Small granules are attached to the widened channels of endoplasmic reticulum (Palade granules) The same granules are seen everywhere in the cytoplasm ER = endoplasmic reticulum 17 000×

3 Muscle fibrils of cardiac muscle and the fibrils of other types of striated muscle exhibit no basic differences The possible exception to this might be the Z bands which connect the individual muscle fibrils of the myocardium but not those of Purkinje s fibers

4 Electron microscopic studies of the capillaries and of the immediately adjoining Rouget cells have revealed several thus far unknown facts For example it was found that the endothelial cells of the capillary contained plasmasomes and an endoplasmic reticulum The capillary wall contained even in protocapillaries always one or more mural slits These slits do not occur only in places where two separate endothelial cells touch but rather they are also found to occur where the margins of a single endothelial cell, surrounding the lumen of the tiniest capillary (protocapillary) overlap We could never discover any special substance (cement) occurring within the capillary wall at the endothelial cell boundaries or in the endothelial slits

Physiologically it is especially noteworthy that the inner surface of the capillary is scalloped and that long ribbon like extensions from the endothelial cells may stretch deep into the blood stream within the capillary lumen (endothelial villi or tentacles)

5 The cardiac nerves have been studied and their location and ultramicroscopic structure suggested certain concepts about the mechanism of the pain in Angina pectoris and the mechanism of axon reflexes Axon endings at muscle fibers of the human ventricle have been found for the first time

6 The results of the electron microscopic investigations of the conductive system (Purkinje s fibers) seem noteworthy as the first of this kind

7 The mature red bloodcells in mammals are the only cells of the body containing no plasmasomes (mitochondria) and no endoplasmic reticulum

8 In chronic heart failure in man a progressive osmiophilic degeneration of sarcosomes has been found and linked to functional disturbances and to the presence of lipofuscine and other lipids in muscle fibers of such hearts

9 Droplets of ultramicrosocpic size have been found in the capillary wall in muscle fibers and in nerve axons They are supposed to present an important type of material exchange in

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FINAL REMARKS

Physiologists and cardiologists will always be suspicious upon hearing conclusions concerning the function of organs which are substantiated by histological pictures only The author himself a physiologist and cardiologist entered upon the extremely difficult field of electron microscopic histology with the greatest of trepidation and only for the express purpose of finding guides, in the ultramicroscopic structure of the heart towards the solution of certain unsolved problems in cardiology While the results of more than ten years of research under the worst financial conditions are not overwhelming they are still encouraging enough to inspire further work The following achievements seem, to the author most worthy of note

1 The understanding of the function of the sarcosomes and a recognition of the importance of their varying quantities in the different types of muscle has provided a first solution to the long standing problem of why the heart can beat throughout a lifetime without requiring an extended period of rest a feat impossible to skeletal muscle Through further comparative studies, we have come to realize that the ability of a muscle to do heavy steady work can be mathematically stated as a function of the number and type of sarcosomes found within its muscle fibers This would seem to hold not only for the varying muscle groups within the same animal but also for the same muscle in different species within the animal kingdom (big mammals like cattle small mammals like the rabbit guinea pig rat mouse bat cold blooded animals like frog fish)

2 Differences in the sarcosomes of the auricle and ventricle of the same heart could be determined

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REFERENCES

ARNOLD JULIUS Über feinere Strukturen und die Anordnung des Glykogens in den Muskelfaserarten des Warmbluterherzens *Zbl allg Path* 20 769 1909

ASHLEY CHARLES A PORTER, KEITH R PHILPOTT DELBERT E and HASS GEORGE M Observations by electron microscopy on contraction of skeletal myofibrils induced with adenosinetriphosphate *J Exper Med* 94 9–20 1951

BACKUS ROBERT C and WILLIAMS ROBLEY C Small spherical particles of exceptionally uniform size *J Appl Physiol* 20 224–225 1949

BAKER ROBERT Observations on muscle fibre structure The swelling of muscle fibres by acids and alkalis *J Anat* 81 259–285 1947

BEAMS H W Studies on the "Golgi apparatus" of insect muscle *Anat Rec* 42 323–334 1929

— EVANS T C JANNEY C D and BAKER W W Electron microscope studies on the structure of cardiac muscle *Anat Rec* 105 59–81 1949

BENNETT H STANLEY Some characteristics of the miofibrils and miofilaments of striated skeletal muscle *Anat Rec* 103 7–8 1949

— and PORTER KEITH R An electron microscope study of sectioned breast muscle of the domestic fowl *Amer J Anat* 93 61–106 1953

— The sarcoplasmic reticulum of striped muscle *J Biophys Biochem Cytol* 2 Suppl 171–174 1956

BENNINGHOFF A Blutgefasse und Herz In *Handbuch d Mikr Anat des Menschen* 6 1 1–232 Berlin 1930

BLUHM M M and SITARAMAYA C An electron microscopic study of the muscle fibers of the diaphragm *Quart J Microsc* 92 323–332 1951

BORYSKO EMIL Recent developments in Methacrylate embedding *I u II J Biophys Biochem Cytol* 2 Suppl 3–14 and 15–20 1956

BORRIES BODO V Die Erweiterung des menschlichen Sehvermogens durch das Elektronenmikroskop *Bergmannische Z Gluckauf* 91 1273–1295 1955

living cells and create many new problems in regard to droplet transfer versus molecular transfer of material in living cells

Many items mentioned in the various chapters and demonstrated in the figures have a physiological meaning which is as yet not elucidated Such items include the inter fibrillar membranes in the muscle fibers, and also straight parallel and concentric lamels, and the variations in their appearance in the different sar*cosomes The uncertainty as to physiological function which sur*rounds these items extends also to the ultramicroscopic canal system, which is found in the cardiac muscle of humans as well as of all other animals investigated to date and in the cytoplasm of the capillary wall (endoplasmic reticulum)

DRAPER, M H., and HODGE, A. J Studies on muscle with the electron microscope I The ultra structure of toad striated muscle *Australian J Exper Biol.*, 27 465–503, 1949
— and — Sub-microscopic Localization of Minerals in skeletal muscle by Internal "Microincineration" within the electron microscope *Nature* 163 576–580 1949
— and — Electron induced Microincineration with the electron microscope I Distribution of residual mineral content in vertebrate striated muscle *Austral J Exper Biol* 28 549–557 1950
DWIGHT THOMAS JR. Structure and action of striated muscular fibre *Proc Boston Soc Nat* 16 119–127 1873
EASTLICK, H L. A cytological study of the Golgi substance of striated muscle of vertebrates *J Morph* 61 399–431 1937
EDWARDS GEORG A DE SOUZA SANTOS PERSIO DE SOUZA SANTOS HELENA LOPES and SAWAYA PAULOS Comparative Electron Microscope study of striated muscle ultra structure *Ciencia e Cultura* 5 27–31 1953
— — — and — Electron Microscope studies of insect muscle I Flight and coxal muscle of Hydrophilus Piceus *Ann entomol Soc* 47 343–354 1954a
— — — and — Electron Microscope studies of insect muscle II Flight and leg muscles of Belostoma and Periplaneta *Ann entomol Soc* 47 459–467 1954b
— — — and — Electron Microscope studies of insect muscle III Variations in ultra structure *Bol fac filos ciences et letras Univ Sao Paulo* 19 391–405 1954
— — — — BRUNNER A JR and RUSKA H Electron Microscopy in the study of insects *Rev brasil entomol* 2 97–104 1954c
— RUSKA HELMUT DE SOUZA SANTOS PERSIO and VALLEJO FREIRE ARISTIDES Comparative cytophysiology of striated muscle with special reference to the role of the endoplasmic reticulum *J Biophys and Biochem Cytol* 2 143–156 1956
ENGELMANN TH W Neue Untersuchungen uber die mikrosk Vorgange bei der Muskelkontraktion *Pflugers Arch Physiol* 18 1–25 1878
FARRANT J L and MERCER E H Studies on the structure of muscle II Arthopodmuscles *Exper Cell Res* 3 553–583 1952
FAWCETT D W and SELBY C C Observations on the fine Structure of the Turtle Atrium *J Biophysic and Biochem Cyt* 4 63–73 1958
FISCHER ERNST Changes during muscle contraction as related to the Crystalline pattern *Biochem Soc Symposia* 3 211–239 1941

—— Fortschritte und Grenzen der Übermikroskopie *Bader Rdsch*, 4/5 200–225 1956

BOWMAN, WILLIAM On the minute structure and movements of voluntary muscles *Philos Trans Roy Soc London* 130 457–502 1840

—— Muscle in TODD's *Cyclopaedia of Anat and Physiol* 3 S 509 London, 1847

BRAUNSTEINER H FELLINGER K and PAKESCH F Über die Struktur der Retikulozyten *Acta Haemat* 16 322–328 1956

BREEMEN See VAN BREEMEN

BRUCE A NINIAN Vasodilator Axon Reflexes *Quart J Exp Physiol* 6 339–354 1913

BRUNNER A JR VALLEJO FREIRE, A and SANTOS P SOUZA Electron Microscopy of thin sections of reticulocytes *Experientia* 12 H 7 255–257 1956

BULLARD H HAYS On the interstitial granules and fat droplets of striated muscle *Amer J Anat* 14 1–46 1912

CAJAL, S RAMON Y Observations sur la texture des fibres musculaires des pattes et des ailes des insectes *Internat Mschr Anat* 5 205–232 253–276 1888

CALLAN H G and TOMLIN S G Experimental studies on amphibian oocyte nuclei I Investigation of the structure of the nuclear membrane by means of the electron microscope *Proc Roy Soc London*, B 137 367–378 1950

CHALFIN DAVID Differences between young and mature Rabbit erythrocytes *J Cellular and compar Phys* 47 215–243 1956

CHAPMAN GEORGE B Electron microscopy of ultra thin sections of insect flight muscle *J Morph* 95 237–262 1954

CHLOPKOW A Zur Frage uber die Struktur des Herzmuskelsarkolemms der Saugetiere *Anat Anz* 61 432–442, 1926

COHNHEIM F Über den feineren Bau der quergestreiften Muskelfaser *Virchows Arch path Anat*, 34 606–622 1865

CLELAND K W and SLATER E C The Sarcosomes of the heart muscle *Quart J Microsc* 94 329–346 1953a

—— and —— Respiratory granules of heart muscle *Biochem J* 53 546–556 1953b

DALTON A J and FELIX MARIE D A comparative study of the Golgi complex *J Biophys Biochem Cytol* 2 79–84 1956

DOBIE W MURRAY Observations on the minute structure and mode of contraction of voluntary muscular fibre *Ann Mag Nat Serie* 2 3 109–119 1849

JORDAN H E Studies on striped muscle structure VI The comparative histology of the leg and wing muscle of the wasp with special reference to the phenomenon of stripe reversal during contraction and to the genetic relation between contraction bands and intercalated discs *Amer J Anat* 27 1–68 1920

— The structural changes in striped muscle during contraction *Physiol Rev* 13 301–324 1933

— Structural changes during contraction in the striped muscle of the frog *Amer J Anat* 55 117–129 1934

— The comparative histology of the striated muscle of the leg, wing and elytra of the Japanese beetle with special reference to the structural changes during contraction and their functional significance *J Morph* 96 513–536 1955

KISCH BRUNO Physiological Aspects of electron histology of heart *Fed Proc* 10 Nr 1 1951

— Electron microscopic histology of the heart New York 1951

— Microscopia Electronica del Musculo Cardiaco Rev Soc mex 12 399–404 1951

— The Sarcosomes a vital part of the heart muscle *Exper Med and Surg* 10 208–236 1952a

— Die Rolle der Sarkosome im Herzmuskel *Pflugers Archiv Physiol* 255 130–133 1952b

— Physiologische Ergebnisse der Elektronenmikroskopie des Herzens Verh Dtsch Ges Kreislaufforschg 18 1–8 Darmstadt 1952c

— Electron Microscopy as applied to cardiology *J Mount Sinai Hosp* 19 606–611 1952d

— The heart rate and electrocardiogram of small animals *Exper Med* 11 117–130 1953a

— La microscopie electronique appliquee à la cardiologie *Arch Mal Coeur* 5 440–445 1953b

— The vital importance of the Sarcosomes in the heart Abstracts of Communications XIII *Intern Physiol Congr* 508–519 Montreal 1953c

— ROBERT REMAK *Transact Amer Philos Soc* 44 227–296 1954

— Studies in comparative electron microscopy of the heart I Pipe fish and Bat *Exper Med* 12 335–360 1954a

— Electron microscopy of the sarcosomes in the heart *Biol Bull* 107 292, 1954b

— Studies in comparative electron microscopy of the heart II Guinea pig and rat *Exper Med and Surg* 13 404–428 1955a

GANSLER H Beitrag zur Ultrastructur der marklosen Nervenfasern *Verh dtsch Ges f Path 41* 345–350 1951

GLHUCHTEN VAN A Etude sur la structure intime de la cellule musculaire striee *Anat Anz* 2 792–802 1887

GERSH, ISIDORE The preparation for frozen dried tissue for electron microscopy *J Biochem biophys Cyt* 2 Suppl 37–43 1956

HAGGQUIST J Gewebe und Systeme der Muskulatur In MOLLENDORFFS *Handbuch der mikroskopischen Anatomie des Menschen II* 1931

HALL C E JAKUS M A and SCHMITT F O An investigation of cross striations and myosin filaments in muscle *Biol Bull*, *90* 32–50, 1946

HAYCRAFT, JOHN BERRY Upon the cause of the striation of voluntary muscular tissue *Quart J Microsc* *21* 307–329, 1881

HEIDENHAIN MARTIN Die kontraktile Substanz in "Plasma und Zelle Eine allgemeine Anatomie der lebendigen Masse Zweite Lieferung VON BARDELEBENS *Handbuch der Anatomie des Menschen* Jena 1911

HENLE JAC *Allgemeine Anatomie* Leipzig 1841

HODGE A J Studies on the structure of muscle III Phase contrast and Electron Microscopy of Dipteran flight muscle *J Biophys and Biochem Cytol* *1* 361–380, 1955

— The fine structure of striated muscle A comparison of insect flight muscle with vertebrate and invertebrate skeletal muscle *J Biophys Biochem Cytol* 2 131–142 1956

— HUXLEY HUGH E and SPIRO DAVID Electron Microscope studies on ultrathin sections of muscle *J Exper Med* 99 201–206 1954

HOFFMANN BERLING HARTMUT and KAUSCHE GUSTAV ADOLF Elektronenmikroskopische Untersuchungen uber den Feinbau der Skelettmuskulatur bei Rana temporaria *Z Naturforschg* *5b* 139–144 1950

HOLMGREN EMIL Über die Sarcoplasmakorner quergestreifter Muskelfasern *Anat Anz* *31* 609–621 1907

HOWATSON, ALLAN F The structure of pigeon breast muscle mitochondria *J Biophys Biochem Cytol* 2 363–368 1956

HURTHLE K Über die Struktur der quergestreiften Muskelfasern von Hydrophilus im ruhenden und tatigen Zustand *Pflugers Archiv Physiol* *126* 1–164 1909

JONES W M, and BARER ROBERT Electron Microscopy of the sarcolemma *Nature* *161* 1012–1013 1948

JORDAN H E Studies on striped muscle structure VI The comparative histology of the leg and wing muscle of the wasp with special reference to the phenomenon of stripe reversal during contraction and to the genetic relation between contraction bands and intercalated discs *Amer J Anat* 27 1–68 1920
— The structural changes in striped muscle during contraction *Physiol Rev* 13 301–324 1933
— Structural changes during contraction in the striped muscle of the frog *Amer J Anat* 55 117–129 1934
— The comparative histology of the striated muscle of the leg wing and elytra of the Japanese beetle with special reference to the structural changes during contraction and their functional significance *J Morph* 96 513–536 1955
KISCH BRUNO Physiological Aspects of electron histology of heart *Fed Proc* 10 Nr 1 1951
— Electron microscopic histology of the heart New York 1951
— Microscopia Electronica del Musculo Cardiaco *Rev Soc mex* 12 399–404 1951
— The Sarcosomes a vital part of the heart muscle *Exper Med and Surg* 10 208–236 1952a
— Die Rolle der Sarkosome im Herzmuskel *Pflugers Archiv Physiol* 255 130–133 1952b
— Physiologische Ergebnisse der Elektronenmikroskopie des Herzens *Verh Dtsch Ges Kreislaufforschg* 18 1–8 Darmstadt 1952c
— Electron Microscopy as applied to cardiology *J Mount Sinai Hosp* 19 606–611 1952d
— The heart rate and electrocardiogram of small animals *Exper Med* 11 117–130 1953a
— La microscopie electronique appliquée a la cardiologie *Arch Mal Coeur* 5 440–445 1953b
— The vital importance of the Sarcosomes in the heart Abstracts of Communications XIII Intern *Physiol Congr* 508–519 Montreal 1953c
— ROBERT REMAK *Transact Amer Philos Soc* 44 227–296 1954
— Studies in comparative electron microscopy of the heart I Pipe fish and Bat *Exper Med* 12 335–360 1954a
— Electron microscopy of the sarcosomes in the heart *Biol Bull* 107 292 1954b
— Studies in comparative electron microscopy of the heart II Guinea pig and rat *Exper Med and Surg* 13 404–428 1955a

— Electron microscopic investigation of the lungs (Capillaries and specific Cells) *Exper Med and Surg* *13* 101–117 1955b

— Ghosts of sarcosomes in the human heart *Exper Med and Surg*, *13* 9–16 1955c

— Progresse nello studio al microscopio elletronico del cuore e dei polmoni *Atti Soc lombard Sci med* *10* 704–711 1955d

— Electron microscopy of the flight muscles of insects *Pubbl, Staz Zool die Napoli* *27* 160–167 1955e

— Electron miscroscopy of the atrium of the heart I Guinea Pig *Exper Med and Surg* *14* 99–112 1956a

— Electron microscopy of the capillary wall *Exper Med and Surg* *14* 113–121 1956b

— Elektronenmikroskopische Untersuchungen am Herzen *Z wiss Mikroskop* *62* 510–520 1956c

— The contribution of electron microscopy to the physiology and pathology of circulation XX *Int Physiol Congr* Brussel 1956d

— The sarcosomes of the heart *J Biophys Biochem Cytol*, *2* 361–362 1956e

— An Electron microscopic comparison of different striated muscles *Exper Med and Surg* *14* 273–285 1956f

— Some electron microscopic observations on erythrocytes *Exp Med & Surg* *15* 83–88 1957a

— Electron microscopy of the capillary wall II The filiform processes of the endothelium *Exp Med & Surg* *15* 89–99 1957b

— The contribution of electron microscopy to the problems of heart failure *Transact Am Coll Card* *7* 209–213 1957c

— Electronenmikroscekopische Untersuchungen des Herzens und der Kapillaren *Dsch Med Wsch* *82* 605–606 634–635 1957d

— Der ultramicroscopische Bau der Capillarwand *Acta Physiol Pharmac Neerl* *6* 334–338 1957e

— Electron microscopy of blood platelets *Exp Med & Surg* *15* 272–288 1957f

— Der ultramikroskopische Bau von Herz und Kapillaren *Darmstadt* 1957g

— Electron microscopy of the lungs in pulmonary edema *Exp Med & Surg*, *16* 17–28 1958a

— Electron microscopy of the lungs III Endothelial cells in the smallest arducts *Exp Med & Surg* *16* 1–16 1958b

— New investigations on cardiac nerves *Exp Med & Surg* *16* 81–95 1958c

— The Mechanism of pain in angina pectoris *Exp Med & Surg 16* 90–98 1958d

— The mechanism of axon reflexes *Exp Med & Surg* 16 221–227 1958e

— Electron microscopic studies on the inner structure of sarcosomes *Exp Med & Surg 17* 1–14 1959

— Electron microscopic investigation of the heart of cattle *Exp Med & Surg* 17 247–261 1959

— A new concept of cardiac failure *The Am J Card* 5 March 1960 In print

— A remarkable kind of cells in the human heart *Exp Med & Surg* 18 78–89 1960

— CAVUSOGLU M and MARANGONI BRUNO Electron microscopy of the human heart *Exper Med and Surg* 17 85–100 1959

— and CAVUSOGLU The ultra microscopic structure of the human heart *Exp Med & Surg* 18 70–77 1960

— GREY C E and KELSCH J J Electron histology of the heart *Exper Med and Surg* 6 346–365 1948

— and PHILPOTT DELBERT E Electron microscopic investigations of the human heart *Exper Med and Surg II* 161–173 1953

— and — Electron microscopy observations on sarcosomes I Insects (Bumble bee) *Exper Med and Surg* 13 63–72 1955

KOELLIKER A *Mikroskopische Anatomie* II/1 S 204 Leipzig 1851

— Einige Bemerkungen uber die Endigungen der Hautnerven und den Bau der Muskeln *Z wiss Zool* 8 311–325 1857

— Über die COHNHEIMSCHEN Felder der Muskelquerschnitte *Z wiss Zool* 16 374–382 1866

— Zur Kenntnis der quergestreiften Muskelfasern *Z wiss Zool* 47 689–716 1888

KRAUSE, W Über den Bau der quergestreiften Muskelfaser (I) *Z Ration Med* 33 265–270 1868

KROGH AUGUST The anatomy and physiology of capillaries New Haven 1930

KUHNE WILLIE *Myologische Untersuchungen* Leipzig 1860

LANGLEY J N On Axon reflexes *J Physiol* 25 364–398 1900

LANZAVECCHIA G Studio al microscopio elettronico sull embriologia del miocardio di pollo *Rendiconti Istit Lombardo di Sc e Lettere* 92 B 209–212 1957

LINDNER, F Die submicroscopische Morphologie des Herzmuskels *Zsch f Zellforschung* 45 702–746 1957

—— Die Submicroscopische Morphologie des Herzmuskels Zsch f Zellfschg 45 702–746 1957

Low Frank N The pulmonary alveolar epithelium of laboratory mammals and man *Anat Rec 117* 241–264 1953

Maley G F and Plaut G W E Yields of oxidative Phosphorylation by heart Mitochondria *J Biol Chem* 205 297–302, 1953

Marcus H Über den feineren Bau des menschlichen Herzmuskels *Z Zell Gewebelehre 2* 203–241 1925

McDougall W On the structure of cross striated muscle and a suggestion as to the nature of its contraction *J Anat Physiol* 31 410–441 1897

Meigs Edward B The structure of the element of cross striated muscle and the changes of form which it undergoes during contraction *Z allgem Physiol* 8 81–120 1908

Melland B A simplified view of the histology of the striped muscle fibre *Qart J Microsc* 25 371–390 1885

Monckeberg J G Über die sogenannten abnormen Sehnenfäden *Verh deutsch path Ges 12* Tagg Kiel 1908 p 160

Morgan Councilman Rozsa George Scent Gyorgyi Albert and Wyckoff Ralph W F Macromolecular arrangement within muscle *Science 111* 201–202 1950

Moore Dan and Ruska H Electron microscope study of mammalian cardiac muscle cells *J Bioph and Bioch Cytol* 3 261–268 1957a

—— and —— The fine structure of capillaries and small arteries *J Biophys and Bioch Cytol* 3 457–462 1957b

Muir A R An electron microscope study of the embryology of the intercalated disc in the heart of the rabbit *J Biophys and Bioch Cytology* 3 193–202 1957

Napolitano L and Fawcett Don The fine structure of brown adipose tissue in the newborn mouse and rat *J Bioph & Bioch Cytology* 4 685–692 1958

Newman Sanford B Borysko Emil and Swerdlow Max Ultra microtomy by a new method *J Res nat Bur Standards* 43 183–199 1949

Palade G E A study of fixation for electron microscopy *J Exper Med* 95 285–298 1952

—— The fine structure of mitochondria *Anat Rec 114* 427–452 1952

—— The endoplasmic reticulum *J Biophys Biochem Cytol* 2 Suppl 85–98 1956

—— Microsomes and ribonucleo protein particles Washington D C 1958

— and PORTER KEITH R The Endoplasmic reticulum of cells in situ *Anat Rec* 112 68 1952

POWERS E L EHRET C F ROTH L E and MINICK O T The internal organization of Mitochondria *J Biophys Biochem Cytol* 2 Suppl 341–346 1956

PEASE DANIEL C and BAKER RICHARD F Sectioning techniques for electron microscopy using a conventional microtome *Proc Soc Exper Biol Med* 67 470–474 1948

— and — The fine structure of the mammalian skeletal muscle *Amer J Anat* 84 175–200 1949

PHILPOTT DELBERT E and SZENT GYORGYI ALBERT The series elastic component in muscle *Biochem. biophys acta* 12 128–133 1953

— Electron microscopic investigation of bumble bee wing muscle *Biol Bull* 167 302 1954

— and SZENT GYORGYI ANDREW G The structure of light meromyosin An electron microscopic study *Biochem biophys acta* 15 165–173 1954

PLAUT G W E and PLAUT K ARMSTRONG Oxidative metabolism of heart *Mitochondria J Biol Chem* 199 141–151 1952

POCHE REINHARD Submicroscopische Beitrage zur Pathologie der Herzmuskelzelle bei Phosphorvergiftung Hypertrophie Atrophie und Kaliummangel *Virchows Archiv* 331 165–248 1958

POCHE REINHARD and LINDNER ERICH Untersuchungen zur Frage der Glanzstreifen des Herzmuskelgewebes beim Warmbluter und beim Kaltbluter *Z Zellforschg* 43 104–120 1955

POLICARD A and BAUD C A Les Structures inframicroscopiques normales et pathologiques des Cellules et des Tissus *Paris Masson & Cie* 1958

PORTER KEITH R The sarcoplasmic reticulum in muscle cells of Amblystoma larvae *J Biophys Biochem Cytol* 2 163–170 1956

— and PALADE G E Studies on the endoplasmic Reticulum III *J Biophys and Bioch Cytol* 3 269–299 1957

RAMSEY R and BLUM J The rate of respiration in erythrocytes II *Quart J Exp Physiol* 22 49 1932

REED R and RUDALL K M Electron microscope studies of muscle structure *Biochim biophys acta* 2 19–26 1948

REGAUD CL and FAVRE M Granulations interstitieles et mitochondries des fibres musculairs striees *Compt rend Acad Sci Paris* 148 661–664 1909

RETZIUS GUSTAF Zur Kenntnis der quergestreiften Muskelfaser *Biol Unters* 1 1–26 1881

— Muskelfibrille and Sarcoplasma *Biol Unters N F*, *1* 51–88 1890

— Die Verbindungen zwischen dem Sarcolemma und den Grund membranen der Muskelfibrillen in bildlicher Darstellung *Biol Unters N F*, *19* 77 1921

RHODIN, J Correlation of ultra structural organization Stockholm 1954

ROLLET ALEXANDER Untersuchungen uber den Bau der quergest reiften Muskelfasern I *Teil Denkschr kais Akad Wiss Math naturwiss Kl Wien 49* 81–132 1885

ROUGET, CH Memoire sur le development de la tunique contractile des vaisseaux *Compt Rend 79* 559 1873

— Sur la contractilite des capillaries sanguines *Compt Rend*, *88* 916 1879

ROZSA, G A SZENT GYORGYI and WYCKOFF RALPH W G The fine structure of myofibrils *Exper Cell Res 1* 194–205 1950

RUDOLPH, G Neuere Anschauungen uber die Verknupfung von Muskel und Nervensystem *Ann Univers Saraviens Medium, IV* 109–143

RUSKA HELMUT Elektronenmikroskopischer Beitrag zur Histologie des Skelettmuskels kleiner Saugetiere *Z Naturforschg 9b* 358–371 1954

— EDWARDS G A and CAESAR A A concept of intracellular trans mission of excitation *Experientia 14* 117–120 1958

RUTHERFORD On the structure and contraction of striped muscular fibre *J Anat and Physiol 31* 309–342 1897

SCHAEFER H Grundprobleme der vegetativen tonischen Innervation *Acta Neurovegetat 4* 201–240 1952

SCHMIDT W J Über die Doppelbrechung der I Glieder der quer gestreiften Myofibrillen und das Wesen der Querstreifung uber haupt *Z Zellforschg 21* 224–242 1934

SCHMITT F O BEAR R S HALL, C E and JAKUS M A Electron microscope and X ray diffraction studies of muscle structure *Ann N Y Acad Sci 47* 799–812 1947

SJOSTRAND F S and ANDERSSON E Electron microscopy of the inter calated discs of cardiac muscle tissue *Experientia 10* 369–370 1954

— Electron microscopy of mitochondria and cytoplasmic double membranes *Nature 171* 30–31 1953

— ANDERSON CEDERGREN E and DEWEY M M The ultrastructure of the intercalated discs of frog, mouse and guinea pig cardiac muscle *J Ultrastructure Res 1* 271 1958

SIEKEVITZ, PH Oxidative phosphorylation in muscle mitochondria and its possible regulation *Ann N Y Acad Sci* 72 500–514 1959

SMITH PHILIP R and COPENHAVER WILFRED M Bailey s Text book of Histology Baltimore 1948

SPEIDEL, CARL CASKEY Studies of living muscles II Histological changes in single fibres of striated muscle during contraction and clotting *Amer J Anat* 65 471–529 1939

SPIRO DAVID The ultra structure of striated muscle at various sarcomere lengths *J Biophys Biochem Cytol* 2 157–162 1956

SLATER E C and CLELAND K W Stabilization of Oxidative Phosphorylation in heart muscle sarcosomes *Nature* 1952 117–118

STUDNITZ GOTTH VON Über die Feinstruktur Kontraktion und Farbbarkeit quergestreifter Arthropodenmuskeln Versuche zur Deutung der Querstreifung des Muskels II *Z Zellforschg* 23 1–23 1936a

— Versuche zur Deutung der Querstreifung des Muskels III Der Glykogenspiegel der A und J Schichten während der Ruhe Kontraktion und Erholung *Z Zellforschg* 23 270–279 1936b

SZENT GYORGYI A Chemical Physiology of contraction in body and heart muscle New York N Y 1953

— General views on the chemistry of muscle contraction *Fortsch Kardiol* S 6–51 Basel 1956

TASAKI, I and MIZUTANI K Comparative Studies on the Activities of the Muscle I *Jap J Med Sci* 10 237 1944

— and TSUKAGOSHI M Id II *Jap J Med Sci* 10 245 1944

THIN G On the structure of muscular fibre *Quart J Microsc* 9 (Neue Ser) 16 251–259 1876

TIGERSTEDT R Die Physiologie des Kreislaufes 2nd Ed Berlin & Leipzig 1923

TSUMURA, NAOTAKA The fine structure of erythrocyte in eel (Anguilla Japonica) studied with electron microscope *The Kurume Medical Journal* 5 152–165 1959

VAN BREEMEN V L Myofibril development observed with the electron microscope *Anat Rec* 113 179–195 1952

— Intercalated discs in the heart muscle studied with the electron microscope *Anat Rec* 117 49–63 1953

WATANABE M T and WILLIAMS C M *Mitochondria in the flight muscles of Insects (I)* *J Gen Physiol* 34 675 1951

— and — Mitochondria in the flight muscles of Insects (II) *J Gen Physiol* 37 71 1953

WATSON M L Staining of tissue sections for electron microscopy II *J Bioph and Bioch Cytol* 4 727–730 1958

WEBER H E Algemeine Anatomie des menschlichen Korpers Vol of 4th Ed of Hildebrants Hdb der Anatomie Braunschweig 1830
WYCKOFF R W G Electron Microscopy Technique and applications New York, 1949

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